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CYTOGENETIC AND BIOCHEMICAL STUDIES ON PURINE-REQUIRING MUTANTS OF *DROSOPHILA MELANOGASTER*

by

Ⓒ Merrie Michal Johnson

A THESIS

SUBMITTED TO THE FACULTY OF GRADUATE STUDIES AND RESEARCH
IN PARTIAL FULFILMENT OF THE REQUIREMENTS FOR THE DEGREE
OF MASTER OF SCIENCE

DEPARTMENT OF GENETICS

EDMONTON, ALBERTA
FALL, 1978

THE UNIVERSITY OF ALBERTA

FACULTY OF GRADUATE STUDIES AND RESEARCH

The undersigned certify that they have read, and recommend to the Faculty of Graduate Studies and Research, for acceptance, a thesis entitled, "Cytogenetic and Biochemical Studies on Purine-Requiring Mutants of *Drosophila melanogaster*", submitted by Merrie Michal Johnson in partial fulfilment of the requirements for the degree of Master of Science.

Dave Nash
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DATED July 27 1978

ABSTRACT

Cytogenetic studies indicate that the purine-requiring auxotrophs of *Drosophila melanogaster*, *pur1-1*, *pur1-2*, and *gua1-1*^{ts} are located within bands 9E1 and 9E3 on the first chromosome.

Radioactive tracer studies on wild-type larvae suggest the presence of hypoxanthine-guanine phosphoribosyltransferase, inosine and guanosine kinases, guanine and adenosine deaminases, xanthine dehydrogenase, and uricase. Hypoxanthine-guanine phosphoribosyltransferase appeared not to be very active with guanine as a substrate, but its activity with hypoxanthine was definitive though modest. GMP reductase activity was found to be almost negligible. Radioactive formate was extensively incorporated into purine nucleotides.

Similar biochemical studies on the purine auxotrophs *pur1-1*, *pur1-2*, and *gua2-1* failed to produce any clear-cut results which would resolve the cause of the auxotrophic phenotype. Evidence was presented which suggests that the *ade2-1* mutant is deficient in guanine deaminase activity.

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LIST OF ABBREVIATIONS

A	adenine
ADP	adenosine diphosphate
All	allantoin
AMP	adenylate
AMPS	adenylosuccinate
AR	adenosine
ATP	adenosine triphosphate
DNA	deoxyribonucleic acid
EDTA	ethylenediamine tetraacetate
FGAR	phosphoribosyl-formylglycineamide
G	guanine
GDP	guanosine diphosphate
Gly	glycine
GMP	guanylate
GR	guanosine
GTP	guanosine triphosphate
H	hypoxanthine
HR	inosine
IMP	inosinate
NAA	nucleic acid adenine
NAD	nicotinamide adenine dinucleotide
NAG	nucleic acid guanine
PRPP	phosphoribosyl pyrophosphate
RNA	ribonucleic acid
SA	succinyl adenosine
UA	uric acid
X	xanthine
XMP	xanthylate
XR	xanthosine

CHAPTER I INTRODUCTION

A The Problem

In recent years a number of mutants of *Drosophila* have been isolated which appear to be deficient in purine metabolism (Falk and Nash, 1974; Naguib, 1976; Nash, unpublished). These mutants do not survive on defined medium without the addition of a purine nucleoside. These workers have suggested that such mutants may have biochemical blocks either in purine biosynthesis *de novo* or in enzymes involved in purine interconversion which leave them without enough purines to survive. Under these circumstances, the purines supplied by the medium were assumed to be utilized by the mutants to synthesize the purine nucleotides needed for survival. These assumptions had never been tested biochemically, however. The necessity of such tests to confirm the hypotheses concerning the bases of the auxotrophy of these mutants prompted the major part of the research described in this thesis.

The map locations of three of the mutants studied, *pur1-1*, *pur1-2*, and *gua1-1*^{ts}, had only been approximated by Falk (1973). Another aspect of the research undertaken as part of this project involved a series of crosses of these mutants to stocks containing overlapping deletions of the region of the first chromosome believed to contain the three mutants. The results of these crosses permit relatively precise localization of the mutations.

Henderson et al. (1977) have pointed out some of the important disadvantages of extrapolating results obtained from enzyme studies on cell extracts to the metabolism of intact cells. It was decided to conduct the biochemical investigation of the purine-requiring mutants on whole larvae, as an intact system, in the hope that the biochemical deficiencies were an intrinsic part of the entire organism. Radioactive tracer compounds were employed to elucidate both the normal pathways of purine metabolism in wild-type *Drosophila*, since this had not been done before, and the ostensibly deficient pathways of the auxotrophs.

B Review of Purine Metabolism

It is generally accepted that the pathway of *de novo* synthesis of IMP, shown in Figure I-1, is universal where such synthesis occurs (Henderson, 1972). Hereafter in this text, "*de novo* synthesis" will refer to this pathway. The first step, the synthesis of phosphoribosylamine from PRPP and glutamine, is catalyzed by PRPP amidotransferase. The enzyme, according to *in vitro* studies, is involved in endproduct inhibition. Although it is widely believed to catalyze the rate-limiting step in the pathway, Henderson (1972) has suggested that much more investigation is necessary before this can be concluded for any intact cell system, and that most evidence in animal cells points to PRPP synthetase as a more likely site of inhibition. He also suggested that the sensitivity of the rate of purine synthesis to substrate concentrations might also be important in regulation of the pathway.

Figure I-2 shows a general scheme of purine interconversion in animal tissues. The relative importance of many of these enzymes is quite variable, depending on the organism or cell type studied. For a detailed discussion of these pathways, see the review by Murray (1971). The enzyme which converts either hypoxanthine or guanine to its

Figure I-1

Pathway of Purine Biosynthesis *de novo*

(From Henderson, 1972)

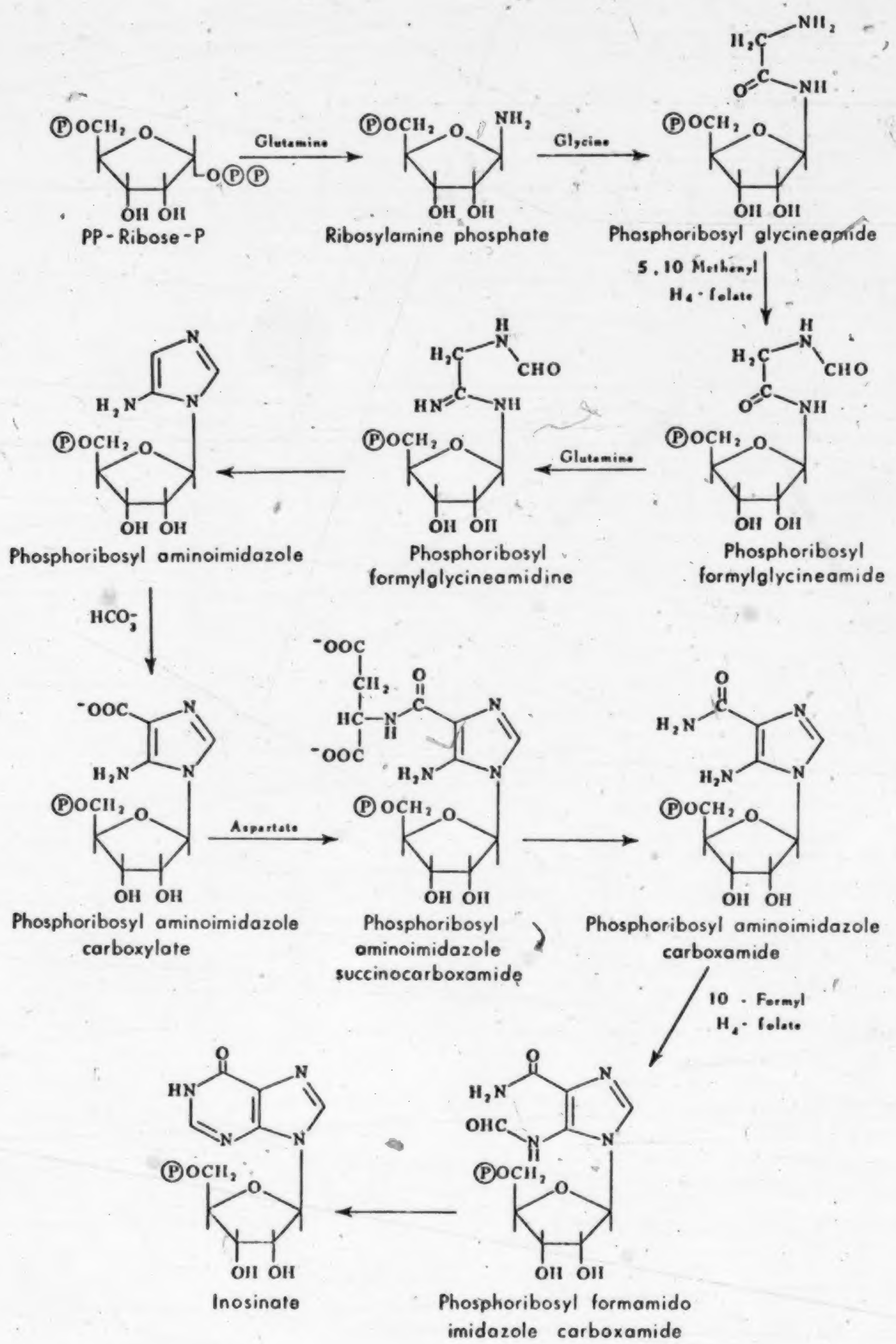
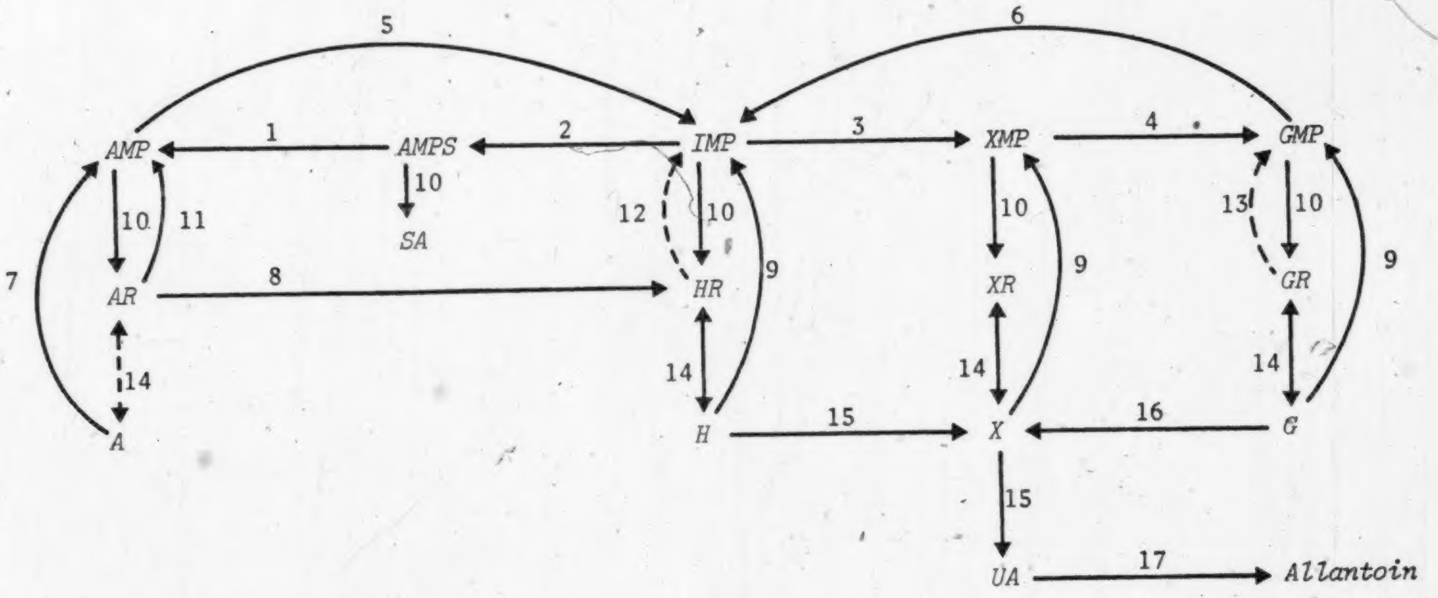

Glutamine
Glycine
PP-Ribose-P
Ribosylamine phosphate
Phosphoribosyl glycineamide
5, 10 Methenyl H$_4$-folate
Phosphoribosyl formylglycineamide
Glutamine
Phosphoribosyl formylglycineamidine
Phosphoribosyl aminoimidazole
HCO$_3^-$
Phosphoribosyl aminoimidazole carboxylate
Aspartate
Phosphoribosyl aminoimidazole succinocarboxamide
Phosphoribosyl aminoimidazole carboxamide
10 - Formyl H$_4$-folate
Phosphoribosyl formamido imidazole carboxamide
Inosinate

Figure I-2

General Scheme of Purine Interconversion in Animal Tissues

(from Miller and Collins 1972, with modification)



1 AMPS lyase
2 AMPS synthetase
3 IMP dehydrogenase
4 XMP aminase
5 AMP deaminase
6 GMP reductase
7 APRT
8 AR deaminase
9 HGPRT
10 5' nucleotidase
11 AR kinase
12 HR kinase
13 GR kinase
14 Purine nucleoside phosphorylase
15 X oxidase
16 6 deaminase
17 Uricase
* Reactions shown with dashed lines have only been shown to have low activity in animal tissues.

appropriate nucleotide is officially called hypoxanthine-guanine phosphoribosyltransferase. However, in referring to one substrate in particular the other name is often dropped, and this convention will usually be followed in this text.

C Biochemical Studies on Purine Metabolism in Dipterans

Purine metabolism in Dipterans has not been widely studied, aside from the extensive work done on *Drosophila* mutants deficient in xanthine dehydrogenase. This work has been reviewed by Dickinson and Sullivan (1975), and will not be discussed here.

Miller and Perry (1968) injected females of *Musca domestica* with ^{14}C-formate and found substantial incorporation of the precursor into purines extracted from ovarian samples. A more extensive study using the same system was performed by Miller and Collins (1973) which revealed several peculiarities of purine metabolism in this organism. Since radioactive adenine was incorporated into both adenine and guanine bases of DNA, they concluded that adenine phosphoribosyltransferase as well as AMP deaminase were present in *Musca* ovaries (assuming that AMP deaminase converted adenine, as AMP, to IMP). The absence of incorporation of radioactive hypoxanthine or guanine into DNA led them to conclude that hypoxanthine-guanine phosphoribosyltransferase was not present in this tissue. On the other hand, radioactive guanosine was readily incorporated into DNA guanine but not adenine, indicating the activity of guanosine kinase and the apparent absence of GMP reductase. Since guanine was not utilized for DNA despite the presence of the kinase, they concluded that guanosine phosphorylase, if present, lacked anabolic activity. However, these workers did not study catabolism of the radioactive compounds, and it may be that a very active guanine deaminase prevented recovery of labelled nucleotides, whether formed via guanine phosphoribosyltransferase or guanosine phosphorylase (and guanosine kinase). They also found that the presence of 6-mercaptopurine riboside, but not its corresponding base, caused sterility of females, lending added support for the conclusion that the phosphoribosyltransferase is absent. Nelson (1964) studied uricase activity at various developmental times in this organism and found activity in larvae and adults a few hours after emergence, but not in pupae or newly emerged adults. He studied extracts of whole eggs, larvae, and pupae, and of Malphigian tubules of adults.

In the discussion of papers which follows, all work has been done using *Drosophila melanogaster*. Becker's work (1974a, 1974b, 1975) has been the most extensive. He studied chiefly extracts of cultured cells, with some experiments on larval imaginal disc and adult extracts of the yellow mutant. He found adenine phosphoribosyltransferase; adenosine kinase; deaminases for AMP, adenine, adenosine, and guanine; inosine and guanosine phosphorylase (base to nucleoside); IMP dehydrogenase; XMP aminase; and xanthine oxidase (in larval discs and adults but not cells). He failed to find activity of hypoxanthine-guanine phosphoribosyltransferase, guanosine kinase, adenosine phosphorylase, or GMP reductase. These findings are similar to those of Miller and Collins (1973) except for guanosine kinase and guanosine phosphorylase. In studies on intact cells (1975), he found that adenosine was not well utilized for nucleic acid synthesis, which he presumed to be due to an active adenosine deaminase coupled with the absence of inosine kinase or hypoxanthine phosphoribosyltransferase. Wagner and Mitchell (1948) used an assay for adenosine deaminase as a measure of growth in their study of *Drosophila* nutrition.

Wyss (1977), on the other hand, in studies of cultured cells found that inosine, adenine, and adenosine could rescue cells grown on methotrexate, which blocks purine synthesis. Since hypoxanthine was not effective in rescueing such cells, he concluded that hypoxanthine phosphoribosyltransferase was absent and that inosine must therefore be salvaged via inosine kinase. He also speculated on the basis of his and Becker's results that purine auxotrophs of *Drosophila* were most likely regulatory mutants, with the supplement required for normal function of feedback systems rather than as a structural component of nucleic acid.

Sensitivity of wild-type *Drosophila* to 6-thioguanine and 8-azaguanine but not to 6-mercaptopurine led Clynes and Duke (1976) to hypothesize the existence of a hypoxanthine-guanine phosphoribosyltransferase different in substrate specificity from the mammalian enzyme. They fed these drugs in a special medium and studied developmental patterns.

Hodge and Glassman (1967) found no adenine deaminase activity in the third instar larval extracts they studied. They determined that guanine deaminase was present in the Pacific wild-type strain, and found no significant conversion of uric acid to allantoin in either the Pacific or the Canton S strains. Seecof (1961) also found substantial guanine deaminase activity in adult extracts.

Morita (1964) studied the enzyme which converts both hypoxanthine to xanthine and xanthine to uric acid and determined that the form present in *Drosophila* is xanthine dehydrogenase, not xanthine oxidase. He found guanine deaminase activity but no appreciable purine nucleoside phosphorylase activity in pupal or adult extracts of the Oregon R strain.

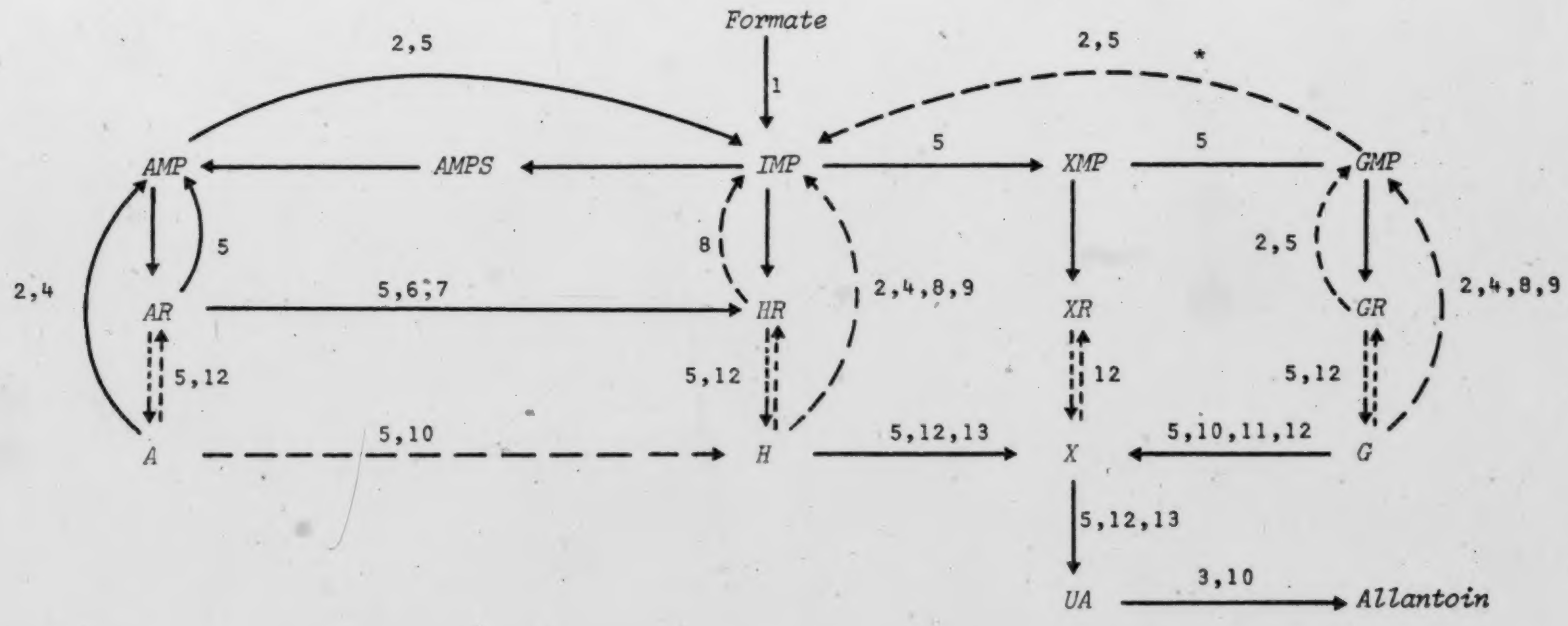
Horikawa et al. (1967), assayed xanthine dehydrogenase activity in cultured embryonic cells of the Oregon R strain. They found that the presence of hypoxanthine in the medium depressed enzyme activity, which they attributed to the presence of more product, since uric acid also inhibited the enzyme.

These results can be summarized by the pathway shown in Figure I-3, which shows the pathways of purine interconversion found in Dipterans. Those which are disputed or thought to be absent are shown in dashed lines, and the references to work done on each enzyme are shown beside the appropriate arrow. For names of the enzymes see Figure I-2.

A large part of the research in the present work involves control studies which shed more light on the activity of many of these enzymes in wild-type *Drosophila*. Because of the diversity of the projects which are part of this thesis, it was decided to include the results, discussion, and conclusions for each part of the research in separate chapters. Thus Chapter III describes the cytogenetic studies, Chapter IV includes the biochemical studies on wild-type *Drosophila*, and Chapter V contains the biochemical studies on the purine-requiring mutants.

Figure I-3

Pathways of Purine Interconversion in Dipterans



[1] Miller and Perry (1968)

[2] Miller and Collins (1973)

[3] Nelson (1964)

[4] Becker (1974a)

[5] Becker (1974b)

[6] Becker (1975)

[7] Wagner and [illegible]tchell (1948)

[8] Wyss (1977)

[9] Clynes and Duke (1976)

[10] Hodge and Glassman (1967)

[11] Seecof (1961)

[12] Morita (1964)

[13] Horikawa et al. (1967)

* Dashed lines indicate either the absence of an enzyme or conflicting results regarding its presence.

CHAPTER II MATERIALS AND METHODS

A Stocks

Cytogenetic mapping experiments were performed using three purine nucleoside-requiring stocks carrying mutations on the X chromosome: *pur1-1*, *pur1-2*, and *gua1-1*^{ts}. These were tested in crosses to four stocks carrying small overlapping deletions close to the region of the X chromosome to which these mutants had been mapped (Falk, 1973). A fourth one of Falk's mutants, *ade1-1*^{sd} and the wild-type stock from which all four mutants were derived were also crossed to the deletion stocks, as controls (the *ade1-1*^{sd} mutant maps in an entirely different region from these mutants). Biochemical analyses were carried out on five purine auxotrophic mutants, three of them mentioned above (*pur1-1*, *pur1-2*, and *ade1-1*^{sd}), and two located on the second chromosome, *ade2-1* (Naguib, 1976), and *gua2-1* (Johnson, Nash and Henderson, 1977). All stocks used are listed and described in Table II-1. Stocks were maintained at 25°C.

B Crosses

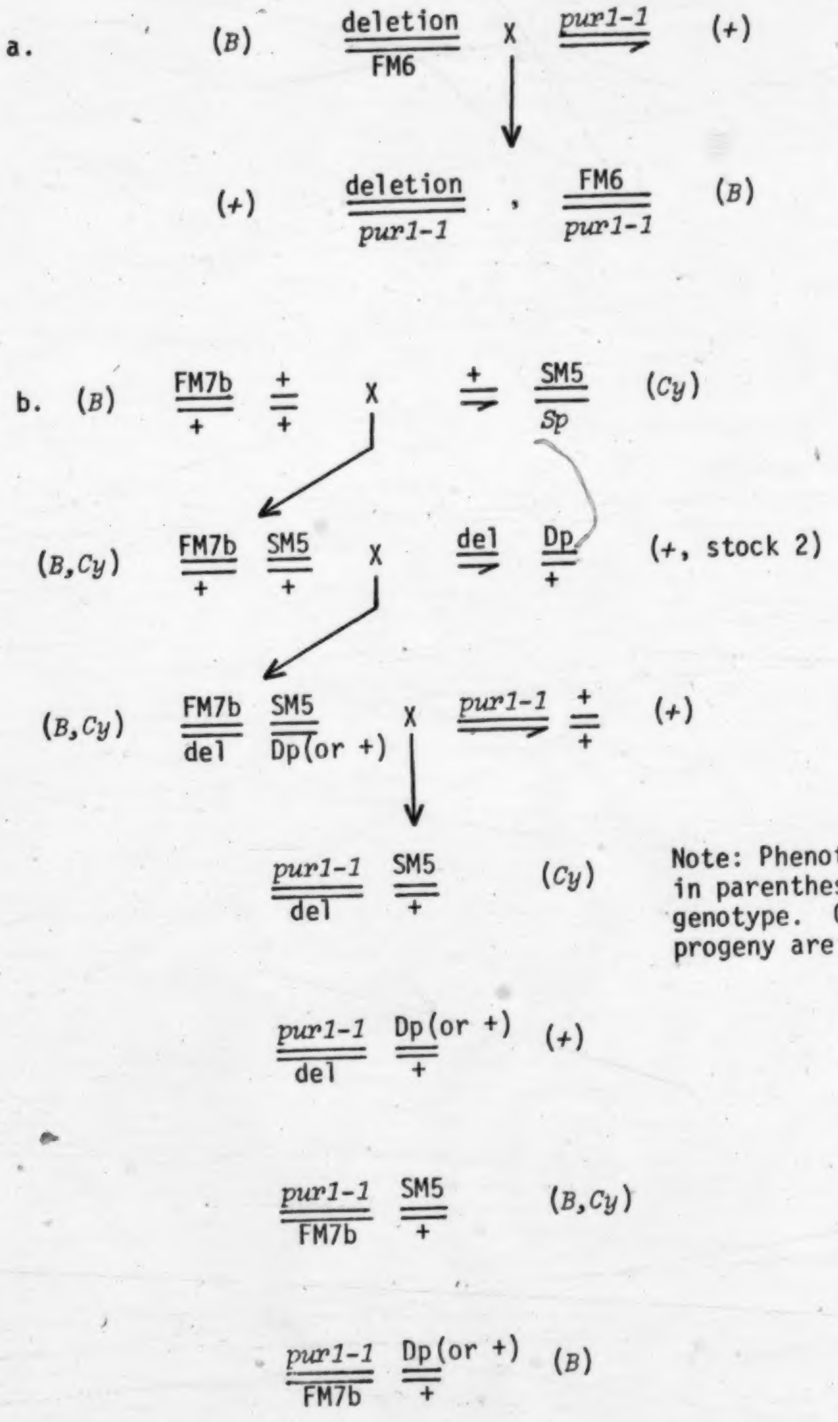
Deletion mapping of three of the mutants above was carried out using the following procedure. Females, heterozygous for the deletion and the dominant mutation *Bar*, were crossed to mutant males. The female progeny were scored for the proportion of mutant/deletion heterozygotes (see Figure II-1a for an example of this cross). With stock number 2, it was necessary to separate the duplication from the deletion in the progeny, so a slightly more complicated series of crosses was required. This is illustrated in Figure II-1b.

Table II-1. List of *Drosophila melanogaster* Stocks Used

	Stock Designation	Origin	Description
(1)	"Amherst" Oregon R.	Amherst College	Inbred derivative of Oregon R (See Stock 1, Amherst College, Drosophila Information Service, 1968).
(2)	Df(1)v^{64f29}/*yw*:=; Dp(1:2)v^{+63i}/+	Lefevre	Df(1)9E4-10A2; Dp(1:2)9E1-10A3. Dp/Dp lethal.
(3)	Df(1)v^{74}/FM6	Lefevre	Df(1)9B1-10A1: FM6 carries *Bar*.
(4)	Df(1)*ras*-$v^{17C.c8}$ In(1)$sc^{S1L}sc^{8R}$+dl-49,$B/ct^{n}oc$	Lefevre	Df(1)9D1-10A3.
(5)	Df(1)c52/FM6	Lefevre	Df(1)8E4-9D3^{+}: FM6 carries *Bar*.
(6)	*pur1-1*	Falk	Purine nucleoside auxotroph supplemented by guanosine and partially by adenosine. Viability about 7% at 25° unsupplemented. X-linked(∿3).
(7)	*pur1-2*	Falk	Allele of *pur1-1*; supplemented completely by guanosine or adenosine. Viability about 3% at 25° unsupplemented. X-linked(∿3).

(8)	$ade1\text{-}1^{sd}$	Falk	Ecloses about 3 days later than wild type on defined medium. Adenosine restores normal development time. Guanosine lowers viability. Pyrimidines have no effect. X-linked(∿57?).
(9)	$gua1\text{-}1^{ts}/yf\!:=$	Falk	Supplemented by guanosine but not adenosine. Almost lethal at 29°; viability about 50% at 25°. Also contains a female sterile mutation and an allele of *white* giving brick red eye colour. X-linked(∿6).
(10)	*ade2-1*	Naguib	Supplemented by adenosine and inosine but not guanosine. Chromosome 2, probably.
(11)	*gua2-1, b*	Nash	Supplemented by guanosine but not guanine, adenosine or inosine. Almost lethal at 25° unsupplemented. Chromosome 2,(∿54).
(12)	FM7b Ins(1)sc^{8}+150-E; 20A-B+d149, $y^{31d}sc^{8}w^{a}lz^{sp}B$	Merriam	X chromosome balancer.
(13)	SM5/*Sp* In(2LR)SM5,$al^{2}Cy\ it^{v},cn^{2},Sp^{2}$	Unknown	Second chromosome balancer.

Figure II-1. Diagrams of Typical Crosses Performed to Map X-linked Purine Auxotrophs


a.
(*B*) deletion / FM6 X *pur1-1* (+)
(+) deletion / *pur1-1* , FM6 / *pur1-1* (*B*)
b. (*B*) FM7b / + + / + X + SM5 / *Sp* (*Cy*)
(*B,Cy*) FM7b / + SM5 / + X del Dp / + (+, stock 2)
(*B,Cy*) FM7b / del SM5 / Dp (or +) X *pur1-1* + / + (+)
pur1-1 / del SM5 / + (*Cy*)
pur1-1 / del Dp (or +) / + (+)
pur1-1 / FM7b SM5 / + (*B,Cy*)
pur1-1 / FM7b Dp (or +) / + (*B*)

Note: Phenotypes are given in parentheses beside each genotype. Only female progeny are shown.

Crosses were carried out axenically on yeast and on Sang's medium (see below) at 25°C and 29°C.

Recombination in the females of the above crosses was suppressed by the presence of inversion on the *Bar* X chromosome. Hence, segregation of *B* from B^+ can be taken as a measure of the segregation of the deletions from their undeleted homologues.

C Axenic Culture

Studies on nutritional mutants require that experiments be carried out free of microbial contamination. This was accomplished by sterilizing the stocks used, raising them on sterile medium, and transferring them in sterile rooms or hoods.

The initial sterilization was carried out on eggs: To collect eggs, adults were fed on live yeast paste for three days, then placed on a 1.5% agar medium to lay for 12 hours. These were sterilized by immersion for 30 minutes in a freshly filtered solution of 3% calcium hypochlorite. The eggs were then removed by filtration, rinsed three times in sterile Ringer's solution (NaCl 7.5g, KCl 0.35g, $CaCl_2$ 0.21 g, H_2O 1000 ml) (Ephrussi and Beadle, 1936), and placed in sterile culture vials.

Stocks were maintained and some experiments carried out on a yeast medium containing antibiotics to prevent contamination (see Table II-2A for composition). Experiments requiring defined medium were carried out on a modification of Sang's (1956) medium (see el Kouni and Nash, 1974; and Table II-2B). Periodically, stocks were checked for contamination by plating a sample of medium on a microbial culture medium (see Table II-2C).

Table II-2. Composition of Media

A. Yeast Medium

Brewer's Yeast	12.50 g	Penicillin*	25,000 I.U.
Sucrose	10.00 g	Propionic acid*	1.00 ml
Granulated agar	2.00 g	Water	90.00 ml
Streptomycin	25.00 mg		

B. Defined Medium

Agar (Oxoid No. 3)	2.50 g	Biotin	0.16 mg
Casein (Vitamin-Free)	5.50 g	Folic acid	0.30 mg
Sucrose	0.75 g	$NaHCO_3$ (anhydrous)	140.00 mg
Cholesterol	0.03 g	KH_2PO_4 (anhydrous)	183.00 mg
Lecithin	0.40 g	K_2HPO_4 (anhydrous)	189.00 mg
Thiamine	0.20 mg	$MgSO_4$ (anhydrous)	62.00 mg
Riboflavin	0.10 mg	*Streptomycin	13.20 mg
Ca Pantothenate	1.60 mg	*Penicillin	25,000 I.U.
Pyridoxine	0.25 mg	Water	to 100 ml

C. Microbial Testing Medium (YEPD)

Agar	1.50 g	Peptone	2.00 g
Yeast Extract	1.00 g	Dextrose	2.00 g
Water	100.00 ml		

* Added after autoclaving

D Chemicals

Radioactive chemicals were all obtained from New England Nuclear. The compounds used, and their specific activities, were as follows: 8-^{14}C guanine (ca. 55 mCi/mmole), 8-^{14}C hypoxanthine (ca. 48 mCi/mmole), 8-^{14}C adenosine (51.2 mCi/mmole), 8-^{14}C guanosine (49.2 mCi/mmole), and 8-^{14}C inosine (ca. 52 mCi/mmole). ^{14}C-Sodium formate had a specific activity of 56 mCi/mmole. The 1-^{14}C glycine used for some control experiments had a specific activity of 47 mCi/mmole, whereas the 1,2-^{14}C glycine used for mutants and other controls had a specific activity of 96 mCi/mmole.

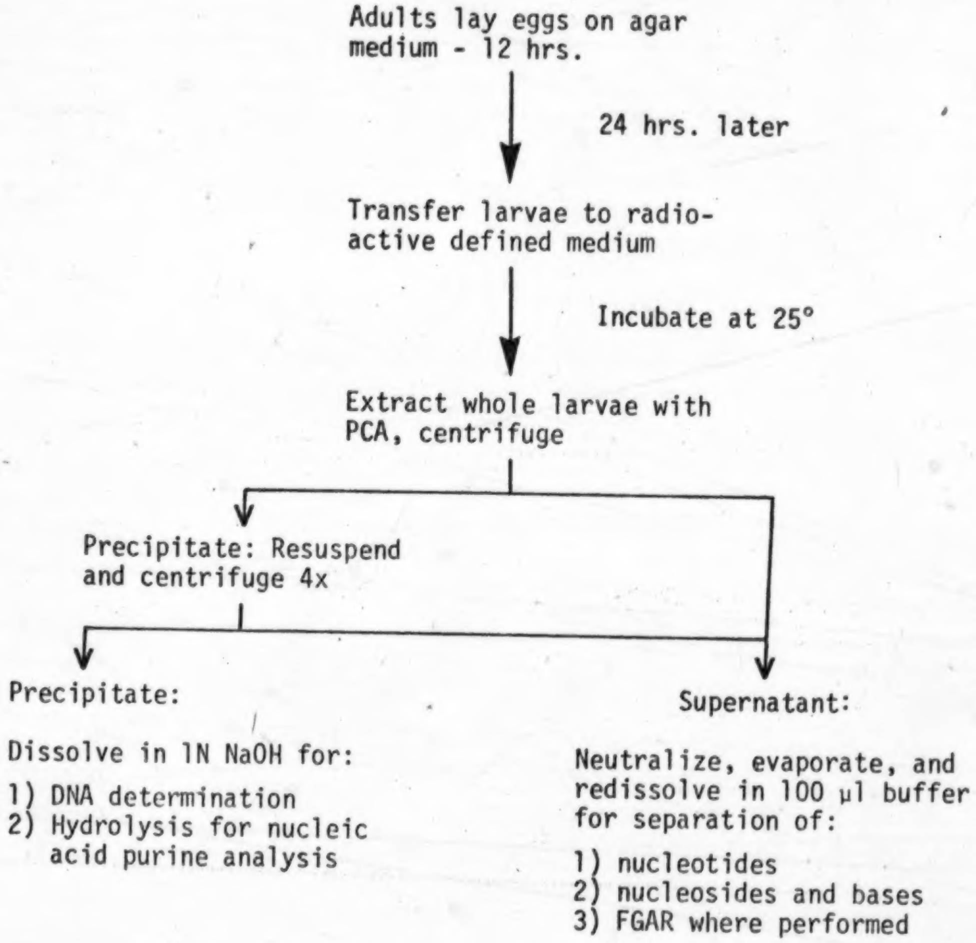
Allopurinol (4-hydroxypyrazolo[3,4-*d*]pyrimidine), and other non-radioactive compounds, adenine, adenosine, guanine, guanosine, hypoxanthine, inosine, xanthine, aminoimidazolecarboxamide, xanthosine, uric acid, AMP, ADP, ATP, GMP, GDP, GTP, IMP, XMP, NAD, AMPS, and allantoin were obtained from Sigma Chemical Company. Azaserine (*O*-diazoacetyl-*L*-serine) was obtained from the Drug Evaluation Branch, Drug Research and Development, National Cancer Institute, Bethesda, Maryland. Succinyl adenosine was prepared by enzymatic dephosphorylation of AMPS (G. Zombor and J.F. Henderson, personal communication).

E Larval Isolation and Incubation

Metabolism of radioactive compounds was measured in larvae following various periods of growth. Figure II-2 presents a general outline of the experimental procedures used.

Cultures with defined media were prepared as follows. A carefully measured amount (0.5 - 1.5 ml) of the radioactive compound to be used was pipetted into a 30 x 12 mm "sealable culture dish" obtained

Figure II-2. Summary of Procedures Used for Biochemical Analyses


Adults lay eggs on agar medium - 12 hrs.
24 hrs. later
Transfer larvae to radioactive defined medium
Incubate at 25°
Extract whole larvae with PCA, centrifuge
Precipitate: Resuspend and centrifuge 4x
Precipitate:
Dissolve in 1N NaOH for:
1) DNA determination
2) Hydrolysis for nucleic acid purine analysis
Supernatant:
Neutralize, evaporate, and redissolve in 100 µl buffer for separation of:
1) nucleotides
2) nucleosides and bases
3) FGAR where performed

from Bellco Glass Company (these dishes will hereafter be referred to as plates). When non-radioactive supplements were added (i.e., to support growth of mutants), these were added to the radioactive solution, which was then evaporated to dryness *in vacuo* to prevent dilution of the medium. The following concentrations of radioactive compounds were used: guanine, hypoxanthine, guanosine, formate, and glycine, 1 mM; adenosine and inosine, 0.3 mM (due to the toxicity of these two compounds). Aminoimidazolecarboxamide and azaserine when used were added at a concentration of 1 mM. Non-radioactive adenosine and guanosine, where used, were added at the usual supplementary dose for mutants, 0.1% or about 3.5 mM (Falk, 1973). The compounds were then redissolved in 50 µl of sterile distilled water and 1 ml of defined medium (at 70°) was pipetted into the plate and stirred. The plate was either used within 24 hours or was stored wrapped in plastic at 4° until about 30 minutes before use.

Eggs were collected as described previously with the substitution of sterile yeast medium for live yeast paste and incubated for 24 hours to allow them to hatch. A specified number of larvae (200-400) were transferred to each plate. A dissecting microscope was used to facilitate transfer of the larvae; a scalpel was used for this operation.

These operations were performed under sterile conditions except for the evaporation, and plates were checked for contamination as described previously. These plates were incubated for four days and samples of larvae were removed for testing either each day or on the second and fourth days.

F Extraction

The number of larvae used for each sample varied depending on the size of the larvae (i.e., approximately 60-90 on day 1 and 2, 30-70 on day 3, and 15-70 on day 4). Usually duplicate analyses were performed. The larvae were transferred from the plate into a 10 x 75 mm glass test tube with a scalpel, using a dissecting microscope. One hundred µl of 0.4 M perchloric acid was added and larvae were then disrupted by sonication, using a Branson Instruments Sonifier, model LG-75. Larval samples were sonicated using 20-30 successive bursts of about one second in duration, at one second intervals, on setting 5. Samples were then centrifuged in an International Clinical centrifuge at full speed for one minute. Sonication and centrifugation were repeated three times without removing the perchloric acid. The supernatant was then pipetted to a clean tube. The pellet was then resuspended, sonicated and centrifuged four times with successive 100 µl aliquots of 0.4 M perchloric acid. These "wash" supernatants were pooled with the original supernatant (referred to as the "extract").

The pH of the supernatants was adjusted to between pH 6.8 and 8.2 with 4.5 M KOH, using phenolphthalein and bromcresol purple; the solution was centrifuged to remove the $KClO_4$ precipitate, and pipetted into a clean tube. These procedures were carried out at 4°. They were then further chilled, evaporated to dryness *in vacuo* over NaOH flakes and stored at -20°. The acid-insoluble fraction was dissolved in 100 µl of 1 M NaOH and left sealed at room temperature for two to five days to hydrolyze the RNA.

G Measurement of DNA

The DNA content of each sample was determined using the fluorometric assay developed by LePecq and Paoletti (1966) as modified by Morgan and Pulleyblank (1974), and further modified by M. Botkin and L. Brox (personal communication). This assay is based on the fact that the increase in the fluorescence of ethidium bromide upon binding to nucleic acid is, under certain conditions, proportional to the amount of nucleic acid present. The reagent solution contained 0.5 ml of ethidium bromide (1 mg/ml), 2.5 ml EDTA (disodium salt, 0.2 M, pH 8), 5.0 ml Tris buffer (1 M, pH 8) and distilled water to make 1 litre. The final pH was adjusted to 8.0 if necessary.

Assays were carried out using a Turner model 430 spectrofluorometer. Excitation and emission wavelengths were 525 and 600 nanometers, respectively. The blank consisted of 2 ml of reagent solution.

A standard solution of high molecular weight calf thymus DNA (50 μg/ml in water) was prepared and diluted 1:1 with 1 M NaOH just before use. Standard curves were obtained by adding successive 10 μl aliquots of the DNA to 2 ml of the reagent solution. In order to keep the pH constant, HCl was always added to neutralize the NaOH in which the DNA was dissolved. Thus for each 10 μl of DNA, 5 μl of 1 M HCl was added. Cuvettes were inverted several times after each addition to obtain a uniform solution, and readings were taken after three minutes.

The DNA content of the acid-insoluble fraction (dissolved in NaOH) of the larval preparations was then determined. Samples were assayed in the manner described above except that 10 μl of 1 M HCl was added per 10 μl of sample.

H Separation of Radioactive Metabolites

1. Nucleic Acid Purines

Nucleic acid adenine and guanine were isolated by adding 10 µl of 7.2 M perchloric acid to the acid-insoluble fraction (dissolved in NaOH) and boiling in a water bath for one hour. After cooling, the samples were centrifuged in a clinical centrifuge at full speed for three to six minutes. The supernatant was then pipetted onto a Dowex-50-H^+ column approximately 20 mm high in 10 mm (I.D.) glass tubing attached to a 50 ml Erlenmeyer flask. The column was washed with 5 ml of 0.1 N HCl and the purines were then eluted with 15 ml of 6 N HCl. The samples were chilled and evaporated to dryness *in vacuo*.

Each sample was dissolved in 200 µl of water, of which 150 µl was applied as a 2 cm streak to Whatman 3 MM chromatography paper. Chromatograms were developed (descending) in isopropanol:2 N HCl (65:35, v/v) for about 24 hours. They were dried, cut into 1 x 2 cm sections, and the radioactivity in each section measured. The Rf of guanine was approximately 0.24 and that of adenine approximately 0.44.

2. Purine Nucleotides

The purine ribonucleotides of the acid-soluble fraction were separated using a one dimensional chromatographic system originally described by Crabtree and Henderson (1971) and slightly modified by Henderson, Fraser, and McCoy (1974). Extracts were dissolved in 100 µl of 25 mM phosphate buffer, pH 7.4. Ten or 20 µl of each extract and about 30 nmoles of each nucleotide carrier were applied to pre-washed polyethyleneimine cellulose thin layers on Mylar sheets (20 x 20 cm, obtained from Brinkman Instruments Ltd.). Plates were then washed overnight in 50% methanol to remove bases and nucleosides.

Successive development in sodium formate buffers (pH 3.4) at the following concentrations gave good separation of NAD, AMP, IMP, XMP, GMP, ADP, GDP, ATP, and GTP: 0.5 M (to 2 cm above the origin), 2 M (to 8 cm above the origin), and 4 M (to the top of the plate).

AMPS was separated when necessary from ADP and other purine nucleotides using a system devised by Zombor and Henderson (personal communication). Samples were spotted on pre-washed PEI cellulose plates with carriers and washed overnight in methanol, as usual. The chromatograms were then developed using 0.8 M NaCl up to 3.5 cm from the bottom of the plates, and 1.25 M NaCl until NAD had nearly reached the wick. The sequence of compounds from top to bottom of the plate was NAD, IMP, AMP, GMP, AMPS, XMP, ADP, GDP, ATP, GTP.

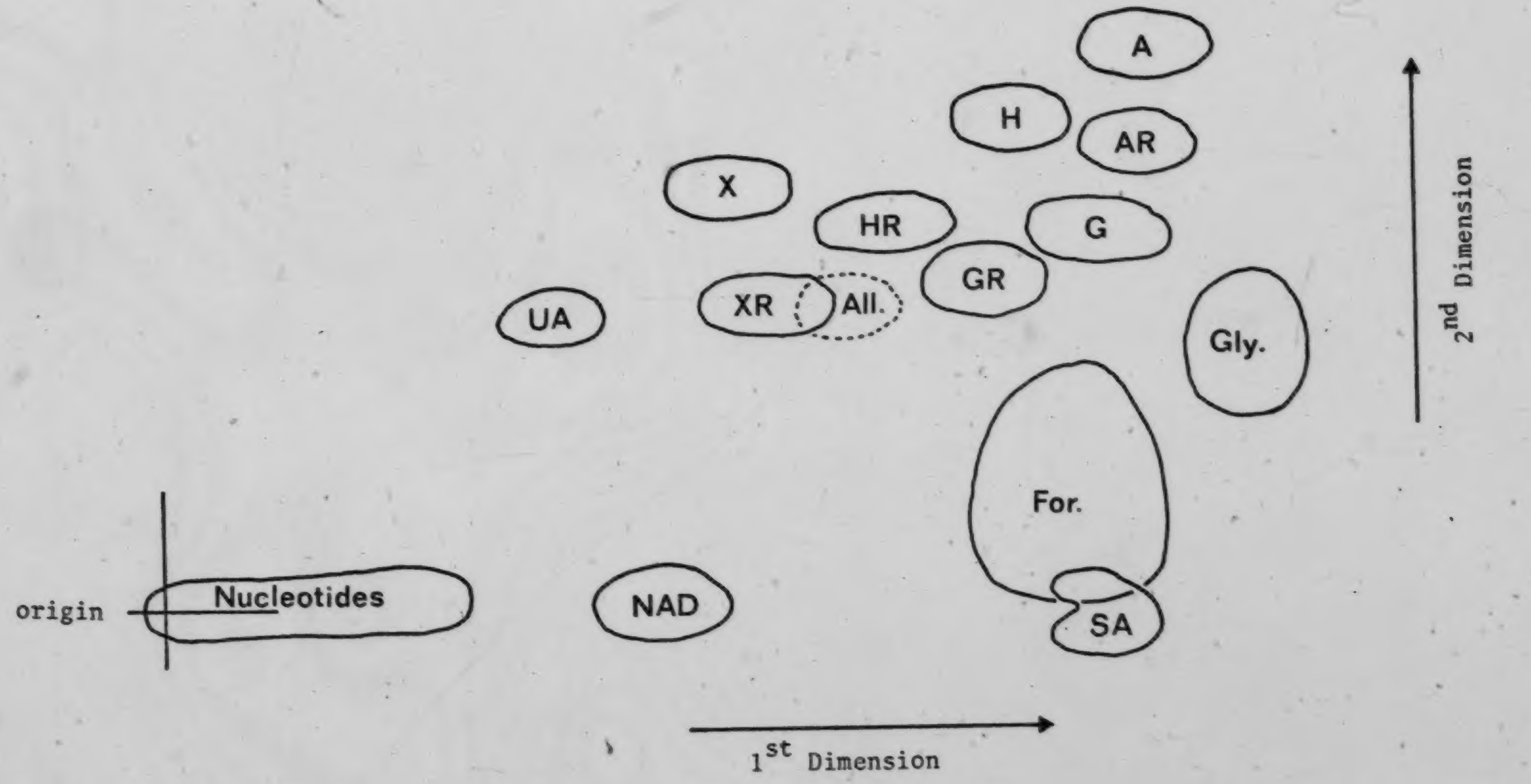
3. Purine Nucleosides and Bases

Purine nucleosides and bases in the acid-soluble fraction were separated with a two dimensional chromatographic system used by Burridge, Woods and Henderson (1977). Polyethyleneimine cellulose sheets, with 28 x 23 cm Whatman 3 MM paper wicks attached to facilitate washing, were washed overnight in 4 M sodium formate buffer (pH 3.4). They were dried and again washed overnight, in 50% methanol and water, then dried and used the same day. Ten μl of sample and the following volumes of carrier solutions were applied to a spot 2 cm from both edges at a corner of the sheet: 5 μl of a nucleotide solution (10 μmoles/ml each of NAD, AMP, IMP, XMP, GMP, GDP, GTP, ADP, and ATP in water); 2.5 μl of a guanine solution (10 μmoles/ml in water); 2.5 μl of a solution of xanthine and uric acid (10 μmoles/ml each in water); 2.5 μl of a solution of hypoxanthine, inosine, adenine, adenosine, guanosine, and xanthosine (10 μmoles/ml each in water); 5 μl of a solution of allantoin (10 µmoles/ml); and 10 µl of a solution of succinyl adenosine (about 2-3 mg/ml). The chromatograms were first developed in 90% formic acid:methanol:water (35:90:15, v/v) until the solvent front reached within 1.0 to 0.5 cm from the wick (about 1-1½ hours at room temperature). The wicks were removed, the plates dried, and developed in the second dimension in 70% 1-propanol until the solvent front was 15-16 cm from the origin (about 3½-4 hours at room temperature). Care was taken to keep the tanks air-tight and free from drafts during the entire time of development. Allantoin was separated from xanthosine in this system by cutting out a clean section of the chromatogram (usually about 3 x 6 cm) containing xanthosine (and allantoin) and developing the "mini"-chromatogram in 50% methanol for about 15 minutes. The ultraviolet absorbing spot was circled and the section was sprayed with a 2% solution of dimethylaminobenzaldehyde in acetone:concentrated HCl (90:10, v/v) in order to visualize the allantoin. Figure II-3 shows the relative positions of the various metabolites on a typical chromatogram.

For a small number of samples not requiring separation of glycine, formate, succinyl adenosine, or allantoin, nucleosides and bases were separated using an ordinary cellulose two dimensional system described in detail in Crabtree and Henderson (1971).

Figure II-3

Relative Positions of Metabolites Separated by PEI
Two Dimensional System



4. FGAR Isolation

Whenever it was necessary to analyze metabolism of the early steps of the *de novo* pathway, larvae were incubated with ^{14}C-glycine and azaserine, which inhibits phosphoribosyl-formylglycineamidine synthetase. Thus ^{14}C-FGAR (phosphoribosyl-formylglycineamide) should accumulate if previous steps in the pathway are not affected by the mutation studied. FGAR was separated from the ^{14}C-glycine using column chromatography (Henderson, 1962). Portions of perchloric acid extracts were poured onto 10 x 40 mm columns of Dowex-1-formate. The effluent was discarded; the column was washed with 30 ml of 0.05 M formic acid, and the FGAR was eluted with 15 ml of 4 M formic acid. Eluates were chilled and evaporated to dryness *in vacuo*. The FGAR was dissolved in 1.0 ml of water.

I Radioactivity Measurements

Ultraviolet absorbing areas of chromatograms were cut out and placed in counting vials with 8 ml of toluene phosphor solution (4 g PPO and 0.1 g POPOP per litre of toluene). Radioactivity in 1 x 2 cm strips of Whatman 3 MM paper from nucleic acid purine separation was measured using 5 ml of phosphor solution. The radioactivity in 0.5 ml of the FGAR solution was measured in 4.5 ml of Bray's solution (Bray, 1960). All radioactivity measurements were made using a Nuclear-Chicago Mark I liquid scintillation counter. Samples were counted for four minutes at a counting efficiency of approximately 60%.

CHAPTER III CYTOGENETIC LOCALIZATION OF *pur1* AND *gua1*

A Introduction

This chapter describes the results of cytogenetic mapping experiments carried out to determine more precisely the location of the *pur1* and *gua1* loci originally approximated by Falk and Nash (1974) as between position 31 and 33 on the X-chromosome. Several stocks containing overlapping deficiencies within this region (obtained from George Lefevre, Jr.) were used in crosses to mutant males. Female offspring heterozygous for a deficiency and a recessive mutation should exhibit mutant phenotype (in this case conditional lethality) if the particular deficiency lacks the region homologous to the mutation. Since the limits of the deficiencies are known, mutations can be mapped quite easily in most cases. The results obtained for the *pur1* locus, while they did permit cytogenetic mapping, were unusual. A complete description of stocks used and diagrams of crosses made are given in Chapter II.

B Control Data

Table III-1 shows the results of crosses between each deficiency and a stock wild-type for the *pur1* and *gua1* loci. In every case except one, this was the "Amherst" stock from which the mutants were derived. The data for defined medium at 29° involves a cross between *Df(1)c52/FM6* and the mutant *ade1-1*^{sd} (which maps at 1-57; see Falk and Nash, 1974). This mutant behaves as does the "Amherst" stock in all other crosses and was used as the control in this case due to insufficient data from the "Amherst" cross.

Table III-1

Survival of Deficiency/Wild-Type Heterozygotes

			Defined Medium		Yeast Medium	
Deficiency	Region Deleted	Segregant Type	25°	29°	25°	29°
v^{64f29}	9E4-10A2	*Df*	204**	163	102	63
		+	193	119	97	77
ras-$v^{17C.c8}$	9D1-10A3	*Df*	379	258*	416	371
		+	451	362*	483	474
v^{74}	9B1-10A1	*Df*	335	70	194	240
		+	238	56	251	287
c^{52}	8E3-9D3	*Df*	90	7	187	85
		+	110	51	218	186

*$ade1\text{-}1^{sd}$ rather than Amherst (see explanation in Section B - Control Data)

**Numbers represent surviving heterozygous female adults

These data clearly show that deficiency heterozygotes are as viable as the wild-type segregants under most condtions. The *ras-v*$^{17C.c8}$ and *c*^{52} deficiency heterozygotes do show some reduction in viability in all cases and this is exaggerated at the higher temperature regardless of medium. On defined medium at 29°, the effect of *c*^{52} is so extreme that the deficiency must be regarded as a dominant semi-lethal; thus the results with *c*^{52} at 29° cannot be used, but are included for completeness.

C Behavior of the *gua1-1*^{ts} Mutant

The *gua1-1*^{ts} mutant is approximately 50% viable on defined medium at 25°, and almost completely lethal on such medium at 29°. The results in Table III-2 indicate that the mutation is exposed by both the *ras-v*$^{17C.c8}$ and the *v*^{74} deficiencies. The deficiency heterozygotes show the expected mutant phenotype on defined medium in both cases, with reduced viability at 25° and near lethality at 29°. As expected, these deficiency heterozygotes survive on yeast medium. On the basis of the results at 29°, the *gua1-1*^{ts} mutation appears to be located between bands 9D1 and 9E3 (omitting, of course, the *c*^{52} data). If the results for the *c*^{52} deficiency at 25° on defined medium are used to extrapolate the expected behavior at 29°, this would narrow the location to between 9D4 and 9E3. This more limited localization can be further refined by the observation that *Dp(1)v*$^{+63i}$ includes *gua1-1*^{ts}. This duplication includes bands 9E1 to 10A11 and in a cross involving males carrying the duplication and females heterozygous for *gua1-1*^{ts} and *FM7b*, 146 *gua1-1*^{ts} males survived on defined medium at 29°, among a total of 701 flies. Since *gua1-1*^{ts} is covered by the

Table III-2

Survival of Deficiency/$gua1\text{-}1^{ts}$ Heterozygotes

			Defined Medium		Yeast Medium	
Deficiency	Region Deleted	Segregant Type	25°	29°	25°	29°
v^{64f29}	9E4-10A2	*Df*	216**	161	180	97
		+	238	151	216	113
ras-$v^{17C.c8}$	9D1-10A3	*Df*	67	9	282	248
		+	316	345	275	328
v^{74}	9B1-10A1	*Df*	72	9	635	328
		+	128	130	594	764
c^{52}	8E3-9D3	*Df*	119	5*	232	57
		+	114	180	264	180

*Also semi-lethal with control

**Numbers represent surviving heterozygous female adults

the duplication, its location is most likely between bands 9E1 and 9E3.

D Behavior of the *pur1* Mutants

Tables III-3 and III-4 show the survival of *pur1*/deficiency heterozygotes. A first glance at the results on defined medium indicates that the *pur1* mutations are exposed by the deficiencies which also expose the *gua1-1*^{ts} mutation, that is, *ras-v*$^{17C.c8}$ and *v*^{74}. Falk and Nash's (1974) approximate map locations are compatible with this conclusion although the cytogenetic localization places all three mutations much closer together than the original mapping experiments might suggest.

The most striking observation about these data, however, is the fact that the relevant mutant/deficiency heterozygotes are of low viability not only on the defined medium, as expected, but also, under certain conditions, on yeast medium. The *pur1-1*/deficiency heterozygotes are of low viability on the permissive yeast medium at both temperatures and the *pur1-2*/deficiency heterozygotes are of low viability on this medium at 29° but not at 25°. This is an unexpected finding since the homozygous (and hemizygous) mutants are in both cases viable on the yeast medium at both temperatures. The simplest explanation for this behavior is that the *pur1* mutants are hypomorphs. That is, the homozygous mutant females (and hemizygous males, which are presumably dosage compensated), are leaky, producing enough functional gene product to support the growth of the flies when purines are present in the medium. This amount is marginal, however, and when it is halved, as with deficiency heterozygotes, the flies show severely reduced viability even with the addition of dietary purines.

Table III-3

Survival of Deficiency/*pur1-1* Heterozygotes

			Defined Medium		Yeast Medium	
Deficiency	Region Deleted	Segregant Type	25°	29°	25°	29°
v^{64f29}	9E4-10A2	*Df*	115**	80	32	47
		+	94	52	40	42
ras-$v^{17C.c8}$	9D1-10A3	*Df*	3	1	0	5
		+	282	431	153	264
v^{74}	9B1-10A1	*Df*	1	0	0	0
		+	47	68	661	351
c^{52}	8E3-9D3	*Df*	123	29*	171	115
		+	158	162	217	330

* Also semi-lethal with control

**Numbers represent surviving heterozygous female adults

Table III-4

Survival of Deficiency/*pur1-2* Heterozygotes

			Defined Medium		Yeast Medium	
Deficiency	Region Deleted	Segregant Type	25°	29°	25°	29°
v^{64f29}	9E4-10A2	*Df*	76**	37	47	24
		+	77	44	42	29
ras-$v^{17C.c8}$	9D1-10A3	*Df*	8	0	64	4
		+	250	103	92	146
v^{74}	9B1-10A1	*Df*	0	0	181	0
		+	158	69	210	344
c^{52}	8E3-9D3	*Df*	95	6*	218	109
		+	109	123	280	244

*Also semi-lethal with control

**Numbers represent surviving heterozygous female adults

Several explanations for the temperature sensitivity of the *pur1-2*/deficiency heterozygotes can be suggested. It is possible that the *pur1-2* mutant produces a slightly temperature-sensitive gene product, which becomes insufficient at the higher temperature. However, since the homozygous mutants do not exhibit a temperature-sensitive lethal phenotype, it must be assumed that the product is not completely inactivated at the higher temperature. Another possibility is that the flies need more purines at 29° and the additional requirement is enough to negate the difference between *pur1-2* and *pur1-1*. This implies that *pur1-1* is somewhat less leaky than *pur1-2*. It is also possible that the *pur1-2* heterozygotes are only marginally viable even with the dietary purines and the general added stress placed on the flies by the higher temperature is enough to kill them.

Since these results are unusual in terms of the expected results of classical deletion mapping experiments, some defense must be made of the claim that the *pur1* mutants have been uncovered by the deficiencies which cause the lethal phenotypes. First, the $gua1\text{-}1^{ts}$ mutant is clearly a classical case; the appropriate deficiency heterozygotes exhibit precisely the $gua1\text{-}1^{ts}$ phenotype. This mutant has already been shown using another technique to map close to the *pur1* mutants; the fact that the same two deficiencies "expose" both the *pur1* mutants and $gua1\text{-}1^{ts}$ thus lends some support to the argument that the *pur1* locus is covered by these deficiencies. Further support is given by studies carried out by Nash (Johnson and Nash, in preparation), who supplemented the lethal *pur1-2*/deficiency heterozygotes on defined medium at 25°. These heterozygotes grew on supplementing concentrations of adenosine and guanosine but not uridine, the same phenotypic pattern shown by the mutants themselves. Since the *pur1-1* mutants are allelic to *pur1-2*, it must be assumed that both are thus exposed by these deficiencies, despite the lethal phenotype on yeast medium shown by *pur1-1* (and *pur1-2* at 29°).

Two similarly anomalous cases have been published. The first was presented by Welshons (1965, 1971), who observed that females heterozygous for facet-notchoid (fa^{no}), (a viable allele of the notch (*N*) locus), and *N* do not survive. He suggested that fa^{no} was a hypomorphic mutation which, in combination with the amorphic *N*, was inviable. The second situation, documented by Lefevre and Johnson (1973), involves a male-viable allele of cut, ct^{71g}, which shows lethality in heterozygous combination with most lethal *ct* alleles. However, in this case the homozygous females are relatively inviable.

E Conclusions

Evidence has been presented in this chapter that pinpoints the location of the $gua1\text{-}1^{ts}$ and *pur1* mutants rather precisely.

(1) All three mutant/deficiency heterozygotes exhibit the mutant phenotype on defined medium with deficiencies $ras\text{-}v^{17C.c8}$ and v^{74}, but not with v^{64f29}, indicating the location to be within 9D1 - 9E3.

(2) Results at 29° on defined medium for the c^{52} deficiency were not used, but results at 25° for both the $gua1\text{-}1^{ts}$ and *pur1* mutants indicate that this deficiency does not cover either locus, narrowing the location to bands 9D4 - 9E3.

(3) Crosses involving $Dp(1)v^{+63i}$ males and heterozygous $gua1\text{-}1^{ts}$ and *pur1-2* females have shown a much higher than normal number of surviving mutant males on defined medium, indicating that this duplication covers the two loci. This information places the final location of the two loci between bands 9E1 and 9E3. Due to technical problems with the c^{52} stock, this is a correction of the earlier location described in abstract form (Johnson and Nash, 1975).

It can be hypothesized on the basis of these data that lethal alleles of the *pur1* locus exist. Similar situations have been described for two other loci, *N* and *ct*.

CHAPTER IV BIOCHEMICAL STUDIES OF WILD-TYPE *Drosophila*

A Introduction

The studies presented in this and the following chapter represent an attempt to define the biochemical bases for the defects of the purine-requiring mutants. It had been previously inferred, from their auxotrophic phenotypes, that the mutations might affect purine metabolism (Falk and Nash, 1974). Since the purine metabolism of wild-type *Drosophila* had not been characterized in detail, it was necessary to include rather extensive experiments on the wild-type as controls. These control experiments are the subject of this chapter.

The techniques used in this study are described in detail in Chapter II. They involve the ingestion of a radioactive compound by the larvae and the "tracing" of the metabolic fate of that compound by the isolation of its various metabolites and the measurement of their radioactivity.

Briefly, newly hatched larvae were transferred to plates containing sterile defined medium to which a radioactive compound had been added. A specific number of larvae were removed for sampling at two and at four days of incubation at 25°. These times were chosen because pilot experiments showed that negligible amounts of the tracer compound were ingested during the first day of larval life; yet, since unsupplemented mutants die as larvae, longer test periods were impractical. The larvae taken for sampling were sonicated in 4% perchloric acid and the nucleic acid fraction separated from the acid-soluble nucleotides, nucleosides, and bases. The radioactivity in these individual metabolites was measured. (See Figure II-2 for summary of procedures used.)

Radioactive adenosine, inosine, guanosine, guanine, and hypoxanthine were used to determine qualitatively and at least semiquantitatively the pathways of interconversion of purine derivatives present in wild-type and mutant *Drosophila*. Radioactive glycine was fed along with azaserine, which blocks the *de novo* pathway at the fourth step, in order to test the intact function of the first three enzymes of the pathway. Radioactive formate was fed along with aminoimidazolecarboxamide (the compound which, as its nucleotide derivative, is needed for the ninth reaction of the *de novo* pathway) in order to test the function of the last two enzymes which synthesize inosinate. Since some of the mutants were believed to be deficient in *de novo* synthesis, the use of glycine and formate would permit testing of at least some of the enzymes of the pathway. More elaborate techniques are necessary to test the other enzymes of the pathway.

The great advantage of these methods is that, being microtechniques, they permit relatively extensive studies of the enzymes of purine metabolism *in vivo*. In some ways this gives a more realistic picture of the actual function of the system of purine synthesis and interconversion than do *in vitro* assays on individual enzymes. There are some drawbacks to the methods, however. Most obviously, they are indirect. Thus, the intermediate steps between precursor fed and the resulting radioactivity in various metabolites must be assumed. In some cases there is little doubt as to which pathway was taken (as in *de novo* synthesis of inosinate). In others there is some uncertainty concerning the relative rates of two or more possible pathways.

Figures IV-1 through IV-6, which illustrate the metabolism of the precursors fed to wild-type larvae, therefore simplify the actual pathways followed by each precursor to some extent. In general, they represent the minimum biochemical interconversion which takes place. Alternative pathways are not shwon, and their elimination is more justified in some cases than in others. Results of work in a variety of systems were used as the basis for construction of the diagrams and further study on *Drosophila* will be required before the assumption of the similarity of this organism to these systems can be evaluated.

Some justification of the elimination of specific pathways is necessary. Figure IV-1, for example, shows that about 4% of radioactive hypoxanthine was converted to adenosine. It is probable that more than 4% was formed, but some was rephosphorylated and some deaminated. Although adenosine deaminase will later be shown to appear quite active when adenosine is used as a precursor, it is not possible to evaluate its activity in most cases and so it is not considered. Adenylate deaminase is not considered, either, in these diagrams, since in no case is its activity distinguishable from that of adenosine deaminase using these techniques.

Inosine can theoretically be formed from hypoxanthine or from IMP, but since ribose-1-phosphate has been found to be not readily available in Ehrlich ascites tumor cells (Barankiewicz and Henderson, 1977), the ribosylation of hypoxanthine (or adenine, xanthine or guanine) has not been considered.

Xanthine will be assumed to be formed from either hypoxanthine or guanine, depending on which precursor was fed.

It is possible that some AMP is formed from adenine via adenine phosphoribosyltransferase. However, evidence in animal tissues suggests that purine nucleoside phosphorylase has a very low activity with adenosine (Murray, 1971; Becker, 1974b), so this latter reaction is not considered. Hypoxanthine-guanine phosphoribosyltransferase will be discussed with each figure as necessary, as will other specific assumptions.

The amount of data generated in this study was very large. Since only a relatively small part of it was directly relevant to the question originally posed (that is: How do the purine mutants differ from wild-type *Drosophila*?), it was decided to include only that part in the body of the thesis. The rest of the data is presented at the end in the Appendix. Wild-type data appear in Tables A-1 through A-5b, which list the radioactivity in counts per minute per microgram of DNA of all the metabolites isolated for each precursor, in the experiments on wild-type *Drosophila* (see List of Tables in Appendix for details). These data have been summarized and presented in Tables IV-3 through IV-6 in this chapter.

B Experimental Conditions and Larval DNA Content

Table IV-1 presents the concentrations of precursors used, the number of larvae and amount of DNA in each sample, and the amount of DNA per larva in each case. The concentration of precursors was 1 mM except for the experiments using adenosine and inosine, where the concentration was only 0.3 mM. The reduction was necessary due to the toxicity of these compounds (el Kouni and Nash, 1977).

With inosine and formate, two separate plates containing larvae were tested in different experiments, and these are indicated as Exp I and Exp II in all tables.

Hypoxanthine was always fed along with allopurinol, which inhibits xanthine dehydrogenase; in early experiments in which radioactive hypoxanthine was fed without allopurinol, about 30% was degraded. Apart from inhibition of degradation, allopurinol appeared to have little effect on hypoxanthine metabolism.

In most cases, duplicate samples were taken on each day. Each replicate sample is indicated by a lower case letter following the day of collection. In cases where duplicate samples were not taken, this was due to a shortage of live larvae on the plate. The number of larvae per sample was also dependent on the number of live larvae remaining on the plate. On Day 2 in the guanosine experiment, there was a large number of dead larvae. A sample made up entirely of dead larvae was taken to test for any obvious differences in the metabolism of guanosine by larvae to which it was toxic. The large number of dead larvae indicate that this was a relatively toxic concentration of guanosine. It was also noted that the larvae which escaped the toxicity, that is, the six still alive on Day 4, were much larger than usual.

Table IV-1

Experimental Conditions and Larval DNA Content--Wild-Type *Drosophila*

Precursor and Concentration	Day and Sample	Number of Larvae/Sample	μg DNA/Sample	μg DNA/Larva
Adenosine	2	50	0.8	0.016
0.3 mM	4a)	41	2.65	0.064
	b)	41	2.4	0.058
Hypoxanthine[a]	2a)	90	1.8	0.020
1 mM	b)	90	1.5	0.017
	4a)	57	2.15	0.038
	b)	60	2.5	0.042
Inosine	2a)	50	0.9	0.018
Exp. I	b)	50	0.9	0.018
0.3 mM	4a)	70	3.4	0.048
	b)	70	3.4	0.048
	c)	70	3.4	0.048
Inosine	2	85	2.2	0.026
Exp. II	4	30	1.6	0.053
0.3 mM				
Guanine	2a)	75	1.45	0.019
1 mM	b)	75	1.7	0.023
	4	53	4.4	0.083
Guanosine	2a)	90	4.15	0.046
1 mM	b)	66(dead)	2.85	0.043
	c)	60	2.0	0.033
	4	6(large)	0.6	0.100

con't.

Table IV-1 (con't)

Precursor and Concentration	Day and Sample	Number of Larvae/Sample	μg DNA/Sample	μg DNA/Larva
Formate[b]	2a)	55	1.0	0.018
Exp. I	b)	50	0.9	0.018
1 mM	4	40	2.2	0.055
Formate[b]	2a)	90	1.8	0.020
Exp. II	b)	60	0.95	0.016
1 mM	4a)	30	1.8	0.060
	b)	31	1.5	0.048

a) 0.5 mM allopurinol added

b) 1 mM aminoimidazolecarboxamide added

The reason for this was not readily apparent.

The differences in DNA content per larva between ostensibly similar samples is most likely due to the fact that the larvae are so small and it is difficult to pick uniform samples of larvae. The larger, more obvious ones are more likely to have been picked for the first sample on a given day.

In general, wild-type larvae show a range of DNA content from 0.016 to 0.026 on Day 2 and from 0.038 to 0.083 on Day 4. The abnormally large size of larvae grown on guanosine (see above) is reflected in exceptionally high DNA contents which fall outside the general range. It appears that while guanosine at the concentration used is demonstrably toxic, it also seems to have a real stimulatory effect on larval growth. In an experiment using a concentration of only 0.7 mM guanosine (not shown in tables), the larval DNA content was only 0.033 μg on Day 2 and 0.052 μg/larva on Day 4.

It is also possible that guanine produces a similar effect, since the next largest amount of DNA per larva on Day 4 was found in larvae fed on guanine. However, since there was no duplicate for this sample and since the Day 2 samples did not show any increase in DNA content, this bears repetition before any such conclusion can be drawn.

The lower value of the range of DNA content on Day 4, 0.038 μg per larva, was recorded for one of the hypoxanthine samples. These two Day 4 samples were both lower than average and it is possible that either the addition of the allopurinol or some interaction between it and the hypoxanthine exerted a slightly inhibitory effect on larval growth.

C Distribution of Radioactivity Recovered

Table IV-2 gives an overview of the fate of each precursor fed to wild-type larvae. Values are intended to illustrate the amount of radioactivity (per unit DNA content) in each sample as well as the fraction which was metabolized. It should be noted that subsequent tables in this chapter show only the percentage of radioactivity found in each metabolite relative to the "Total Amount Metabolized" given in this table. Where formate was used as a precursor, the "Total Amount Metabolized" refers only to radioactivity found in purine derivatives.

Perhaps the most surprising feature of these data is that, regardless of the fact that adenosine and inosine were present in about one-third the concentration of the other precursors, almost all of the samples show approximately the same amount of radioactivity recovered, roughly between 100,000 and 200,000 cpm/μg DNA. Since the specific activities of all the compounds were similar, this means that adenosine and inosine were taken up by the larvae with roughly three times the efficiency of the uptake of the other compounds. It is obvious that the larvae eat enough of the medium (and therefore, in most cases, of the precursor) to accumulate the amount of radioactivity found in the adenosine and inosine samples. Since there is no reason to believe that *Drosophila* larvae eat more or less medium depending on the precursor it contains, it is necessary to assume that the larvae excrete more of other compounds (either metabolized or unchanged) than they do of adenosine and inosine. The apparently preferential uptake of these compounds by the larvae may bear some relationship to their toxicity; just what that relationship could be remains to be discovered.

Table IV-2

Distribution of Radioactivity Recovered in Wild-Type Larvae

			Counts per minute/µg DNA			
Precursor	Day and Sample	µg DNA/ Sample	Total Radioactivity Recovered	Unused Precursor	Total Amount Metabolized	
Adenosine	2	0.8	229,687	45,540	184,147	(80%)[d]
	4a)	2.65	173,498	48,637	124,861	
	b)	2.4	190,675	61,597	129,078	
			182,087[c]	55,117	126,969	(70%)
Hypoxanthine[a]	2a)	1.8	147,340	113,801	33,539	
	b)	1.5	171,347	134,960	36,387	
			159,344	124,381	34,963	(22%)
	4a)	2.15	318,716	219,909	98,807	
	b)	2.5	231,760	158,404	73,356	
			275,238	187,387	87,851	(32%)
Inosine	2a)	0.9	133,767	60,851	72,916	
Exp. I	b)	0.9	165,689	73,996	91,693	
			149,728	67,423	82,305	(55%)
	4a)	3.4	200,215	30,370	169,845	
	b)	3.4	247,503	60,854	186,649	
	c)	3.4	257,465	69,452	188,013	
			235,061	53,559	181,502	(77%)

Inosine	2	2.2	109,568	50,739	58,829	(54%)
Exp. II	4	1.6	197,169	62,428	134,741	(68%)
Guanine	2a)	1.45	110,083	43,629	65,765	
	b)	1.7	86,047	31,760	54,287	
			98,065	37,695	60,026	(61%)
	4	4.4	52,955	19,418	33,889	(63%)
Guanosine	2a)	4.15	135,843	90,239	45,604	
	b)[e]	2.85	169,263	94,620	74,643	
	c)	2.0	150,565	96,087	54,478	
			151,890	93,649	58,242	(38%)
	4	0.6	176,716	101,384	75,332	(43%)
Formate[b]	2a)	1.0	146,690	77,770	68,920	
Exp. I	b)	0.9	163,755	97,086	66,669	
			155,223	87,428	67,795	(44%)
	4	2.2	182,336	73,637	108,699	(60%)
Formate[b]	2a)	1.8	95,922	35,863	60,059	
Exp. II	b)	0.95	119,211	16,999	102,212	
			107,567	26,432	81,135	(75%)
	4a)	1.8	154,411	69,769	84,642	
	b)	1.5	148,653	69,532	79,121	
			151,532	69,650	81,882	(54%)

a) 0.5 mM allopurinol added

b) 1 mM aminoimidazolecarboxamide added

c) Underlined numbers are the means of the two or three preceding samples

d) Percent of Total Radioactivity Recovered

e) All larvae in this sample were dead

The amount of radioactivity recovered from guanine samples was generally lower than that from the other compounds. Two possible explanations for this finding may be suggested. It is quite likely that since the solubility of guanine is much lower than that of the other precursors, this may have affected its uptake by the larvae. An alternative possibility is that it may have been excreted at an increased rate, since it is quite extensively degraded (see discussion of Table IV-6).

The proportion that was metabolized varied considerably from one precursor to another. The lowest fraction metabolized was found in the hypoxanthine samples, in which hypoxanthine degradation was prevented by allopurinol. Relatively little guanosine was metabolized, a fact which may be due to a peculiarity of *Drosophila* metabolism which will be discussed later.

Relatively high proportions (greater than 50%) of adenosine, inosine, and guanine were metabolized. One sample from the formate experiments (Exp II, Day 2, sample b) was exceptionally high, but generally 40 - 60% of formate was found in purine derivatives.

The fact that there were considerable amounts of unused precursor in all cases indicates that the larvae were fed enough of all the precursors to saturate the enzyme systems involved in their metabolism.

D Metabolism of Radioactive Hypoxanthine and Inosine

Table IV-3 summarizes the metabolic fate of hypoxanthine and inosine fed to wild-type larvae for two days. The complete data for these experiments, including Day 4 samples, are shown in the Appendix. Use of Day 2 data is justified by the fact that Day 4 data differ from them only quantitatively and not qualitatively.

When hypoxanthine was fed, 26% of the radioactivity metabolized was found in nucleic acid purines. Most of the radioactivity metabolized (58%) was recovered in acid-soluble nucleotides. The fact that substantial amounts of hypoxanthine were converted to nucleotides supports the view that *Drosophila* larvae may possess considerable hypoxanthine phosphoribosyltransferase activity. This is in contrast to published reports on *Drosophila* cultured cells (Becker, 1974a, 1974b; Wyss, 1977), and on *Musca domestica* ovarian extracts (Miller and Collins, 1973). It is conceivable that IMP might be formed via the activity of purine nucleoside phosphorylase and inosine kinase. Becker (1974b) found activity of the former but not the latter. Morita (1964) found no appreciable activity of the former; however, since the evidence for all three of these enzymes is tenuous, in the interest of simplicity, conversion of hypoxanthine to IMP will be assumed to take place via phosphoribosyltransferase activity.

Very little of the precursor was catabolized, since only about 6% of the radioactivity was recovered in xanthine, uric acid, and allantoin. This is an artifically low finding brought about by the deliberate inhibition of xanthine dehydrogenase by allopurinol. A possible explanation for the difference in the results reported here and those of other workers concerning hypoxanthine-guanine phospho-

Table IV-3

Metabolism of Radioactive Hypoxanthine and Inosine in Wild-Type Larvae[a]

Metabolite	Percent of total amount metabolized		
	Hypoxanthine	Inosine	
		Exp. I	Exp. II
Nucleic Acid Adenine[b]	16	27	32
Nucleic Acid Guanine[b]	10	14	21
AMP + ADP + ATP	47	37	31
GMP + GDP + GTP	6	9	4
IMP	2	3	2
XMP	0.9	1	0.6
AMPS	2	b	0.7
Adenosine	3.7	0.2	0.1
Inosine	3		
Xanthosine	0.2	c	0.1
Guanosine	0.6	1	0.3
Succinyl Adenosine	1	c	0.1
Adenine	0.4	0.8	0.2
Hypoxanthine		3	2
Xanthine	5	0.8	0.7
Guanine	0.9	0.6	0.04
Uric Acid	0.6	0.5	2
Allantoin	0.2	c	3
Allantoin + Xanthosine	c	2	c

a) Only Day 2 data shown

b) Nucleic acid adenine/guanine: Hypoxanthine = 1.7
Inosine (Exp. I) = 1.9
Inosine (Exp. II) = 1.5

c) Not measured

ribosyltransferase may be that no inhibitor was used in other studies.

The ratio of nucleic acid adenine to nucleic acid guanine was 1.7, which is approximately average for most of the precursors studied. It approaches the expected ratio of 1.5 based upon a 40% GC content of *Drosophila melanogaster* DNA and ribosomal RNA (Laird, 1973). In contrast, the ratio of adenine nucleotides to guanine nucleotides in the acid-soluble pool was much higher.

Figure IV-1 shows the pathways involved in hypoxanthine metabolism. Percentages shown are cumulative (as in all figures in this chapter), from the ends of the pathways toward the original precursor. Thus the number 67.1% between "Adenylosuccinate" and "Adenine Nucleotides" includes the fraction of radioactivity found in adenine, adenosine, adenine nucleotides, and nucleic acid adenine.

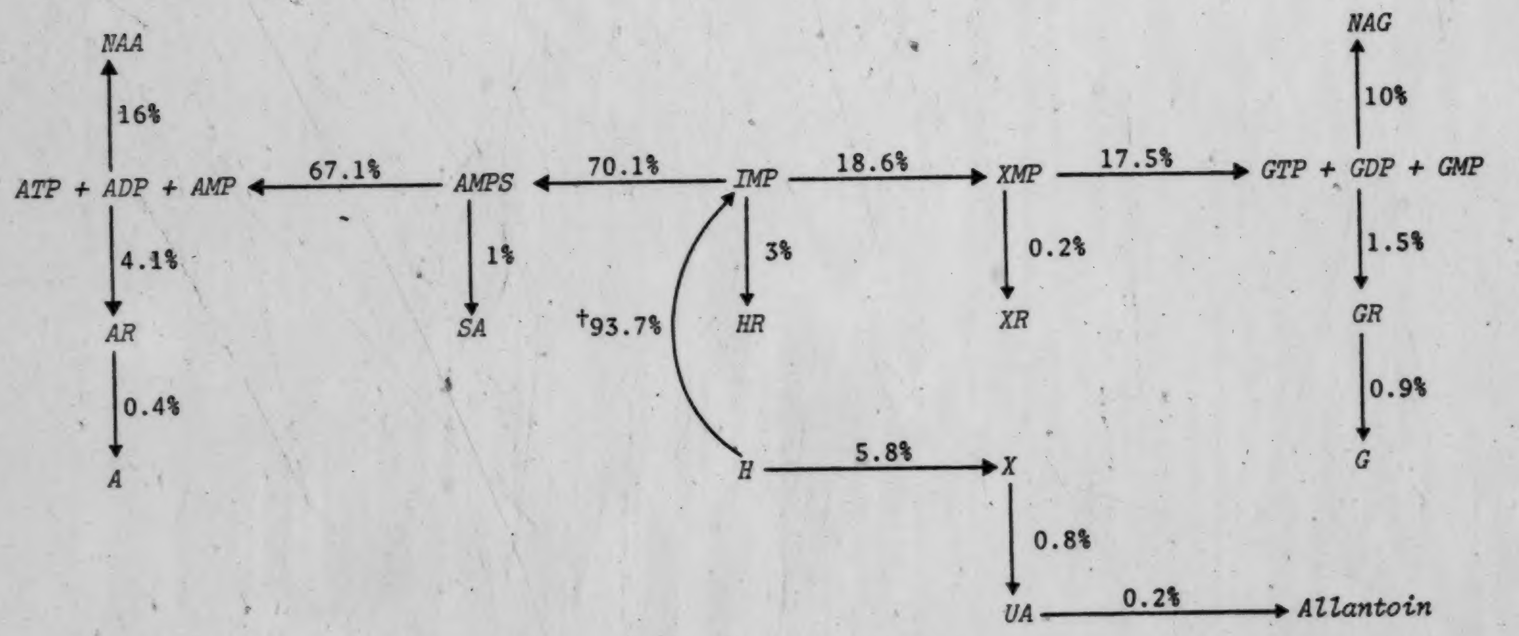
From Figure IV-1, it is apparent that almost 95% of the radioactive hypoxanthine metabolized in the presence of allopurinol was converted to IMP, with only a small amount degraded. Most of the IMP formed was converted to adenine nucleotides, more than three times as much as was converted to guanine nucleotides.

At the other two main branch-points of the pathway, adenine nucleotides and guanine nucleotides, the main direction of metabolism appears to be anabolic. Four times as much of the radioactivity was found in nucleic acid adenine than in adenosine or adenine, and about five times as much of the radioactivity in guanine nucleotides was converted to nucleic acid than was converted to guanosine.

A larger proportion of the radioactivity which was converted to guanine nucleotides was subsequently incorporated into nucleic acids than the proportion which was converted to adenine nucleotides. The

Figure IV-1

Pathways Involved in Hypoxanthine Metabolism



† Percentages are of the "Total Amount Metabolized" for the mean of Day 2 samples, or 34,963 cpm/µg DNA, which is 22% of the "Total Radioactivity Recovered" (see Table IV-2).

larger fraction remaining in acid-soluble adenine nucleotides presumably reflects the fact that these nucleotides have a relatively more important role in energy metabolism than guanine nucleotides.

Two experiments were performed using inosine as a precursor. In Exp II, AMPS was separated from the other nucleotides with a different chromatographic technique than that used in Exp I (see Chapter II, Section H-2). Thus, in the first experiment, the radioactivity in AMPS is partially included with the adenine nucleotides, since it was not separated from ADP. However, since the fraction in AMPS was found to be negligible in Exp II, this has no important effect on the results.

In these two experiments 55 and 54% of recovered radioactivity were identified in metabolic products of inosine. In Exp I, 41% and in Exp II, 53% of the radioactivity metabolized was recovered in nucleic acid purines; 46 and 35% respectively were found in the acid-soluble nucleotide pools.

The fraction remaining in nucleic acid plus acid-soluble nucleotides was similar for both the hypoxanthine and inosine experiments (about 92%). However, since a much larger amount of radioactive inosine was metabolized, this means that the actual amount of radioactive precursor converted to nucleotides was much greater in the inosine experiments.

It is notable that, despite the absence of allopurinol, very little inosine was degraded to xanthine, uric acid and allantoin. Indeed, the immediate product of phosphorolysis of inosine, (namely hypoxanthine) was not found in large amounts (2 - 3%). These findings suggest that inosine may be utilized directly by phosphorylation, rather than degraded first to hypoxanthine, which then acts as a substrate for hypoxanthine phosphoribosyltransferase.

Figure IV-2 illustrates the metabolism of inosine, with separate pathways shown for each experiment. In Exp I, AMPS and succinyl adenosine were not separated and so are not included in the figure. Xanthosine was not separated from allantoin in this experiment. However, since in Exp II where they were separated, most of the radioactivity was found in allantoin, the combined percentage is shown in allantoin in this figure.

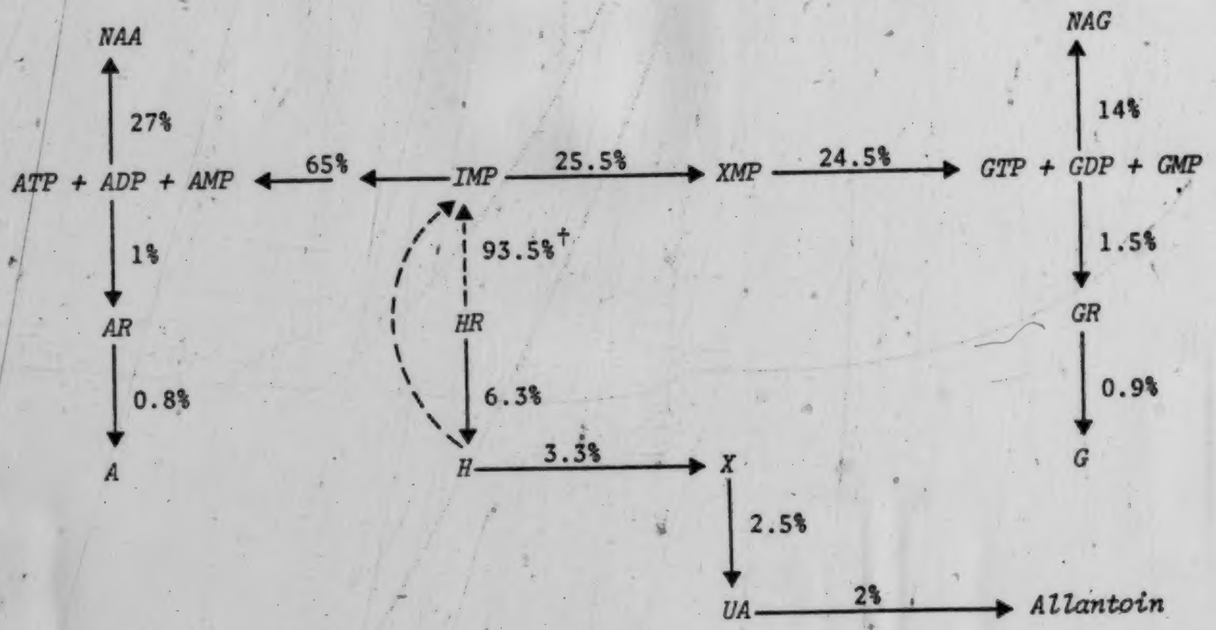
If the two pathways are compared, it can be seen that the results of the two experiments are quite similar. In both experiments, about 93% of the radioactive inosine was converted to IMP and about 7% was degraded. In both cases, about 65% of IMP formed was converted to AMPS and about 26% to XMP and their metabolites. The fractions which were converted to nucleic acid were somewhat higher in Exp II, and there was less catabolism of both adenine and guanine nucleotides.

The main point of ambiguity regarding inosine metabolism concerns the question of whether it is converted directly to IMP via inosine kinase, or whether it is first converted to hypoxanthine and thence to IMP via hypoxanthine phosphoribosyltransferase. As previously mentioned, the lack of degradation of inosine in the absence of allopurinol suggests the possible activity of inosine kinase. There is little evidence for the presence of this enzyme in animal cells (Murray, 1971), however. For this reason, it has been suggested that the lack of degradation found when inosine is used as a precursor may be explained by the possibility that inosine is only relatively slowly converted to hypoxanthine, perhaps giving the phosphoribosyl-

Figure IV-2

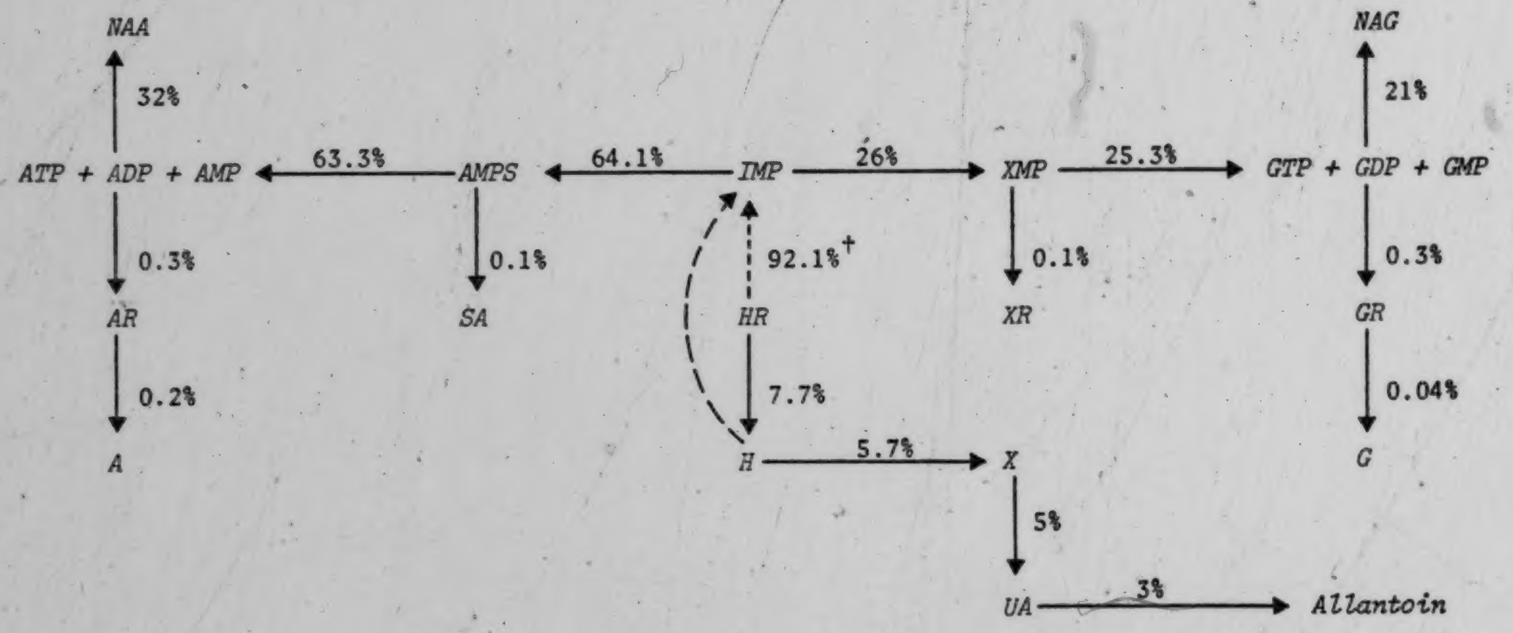
Pathways Involved in Inosine Metabolism

Exp I



† Percentages are of the "Total Amount Metabolized" for the mean of Day 2 samples, or 82,305 cpm/μg DNA, which is 55% of the "Total Radioactivity Recovered" (see Table IV-2).

Exp II



† Percentages are of the "Total Amount Metabolized" for the Day 2 sample, or 58,829 cpm/μg DNA, which is 54% of the "Total Radioactivity Recovered" (see Table IV-2).

transferase a competitive advantage over xanthine dehydrogenase (J.F. Henderson, personal communication). This might be brought about if the concentration and also the K_m of the former were lower than the latter. However, Wyss (1977) found that inosine but not hypoxanthine could rescue cells whose purine synthesis had been blocked by methotrexate. He concluded that inosine kinase but not hypoxanthine phosphoribosyltransferase was present.

Although it is impossible to make a definitive statement regarding either the presence or absence of the kinase on the basis of the data presented, at least one other piece of circumstantial evidence points to its possible presence. Inosine was utilized to a much greater extent than hypoxanthine for nucleotide synthesis, even when degradation of the latter was prevented. A comparison of the fractions of the "Total Radioactivity Recovered" (rather than of the "Total Amount Metabolized") which were converted to nucleotides may serve to clarify this issue. About 50% of the radioactivity recovered when inosine was used as a precursor was converted to nucleotides. This fraction in the hypoxanthine samples was only 20%. This finding could also be explained in the absence of inosine kinase, however, if transport of nucleosides into the cell were more efficient than that of bases. If, as has been suggested, the toxic effect exerted by nucleosides occurs only after conversion to the appropriate nucleotide, the fact that the nucleosides are more toxic than their corresponding bases (with the exception of adenine---see el Kouni and Nash, 1977) would be another indication of the more efficient utilization of nucleosides by *Drosophila* larvae. However, it may be that the nucleosides themselves, rather than their nucleotides, exert the toxic effect (J.F. Henderson, personal communication). Accordingly, since there exists real doubt as to the way in which inosine is metabolized, the alternative pathways are shown in dashed lines in the two figures.

E Metabolism of Radioactive Adenosine

Table IV-4 shows that radioactive adenosine was incorporated into nucleic acid with about the same efficiency as was inosine (about 38%), and with greater efficiency than hypoxanthine. The percentage metabolized that was found in acid-soluble nucleotides was lower than when inosine and hypoxanthine were used as precursors. If the fraction of radioactivity recovered (rather than of the amount metabolized) which was converted to nucleotides is calculated, however, it closely approximates those of inosine samples, or about 50%. This appears to be due to the fact that, although a higher fraction of adenosine than inosine was metabolized, 34% of the amount metabolized represented catabolism to inosine, and somewhat less remained unused when adenosine was used as a precursor.

The high fraction of radioactivity recovered as inosine, 34%, suggests that *Drosophila* larvae have a very active adenosine deaminase. Becker (1974b), working with *Drosophila* cell extracts, also found considerable adenosine deaminase activity. Other than conversion to inosine, there was very little catabolism, with less than 1% of the radioactivity metabolized found in almost any of the other nucleosides or bases.

The ratio of radioactive adenine to guanine in the nucleic acid fraction was 1.6, very close to the same value obtained for hypoxan-

Table IV-4

Metabolism of Radioactive Adenosine in Wild-Type Larvae[a]

Metabolite	Percent of total amount metabolized
Nucleic Acid Adenine[b]	24
Nucleic Acid Guanine[b]	14
AMP + ADP + ATP	19
GMP + GDP + GTP	2
IMP	0.8
XMP	0.5
Inosine	34
Xanthosine	c
Guanosine	0.9
Adenine	0.9
Hypoxanthine	0.9
Xanthine	0.1
Guanine	0.6
Uric Acid	0.7
Allantoin + Xanthosine	2

a) Only Day 2 data are shown

b) Nucleic acid adenine/guanine = 1.6

c) Not measured

thine, inosine, and formate samples. This ratio in acid-soluble nucleotides was almost 10, which is somewhat higher than the 8 found in hypoxanthine samples and the average of 6 found in inosine samples.

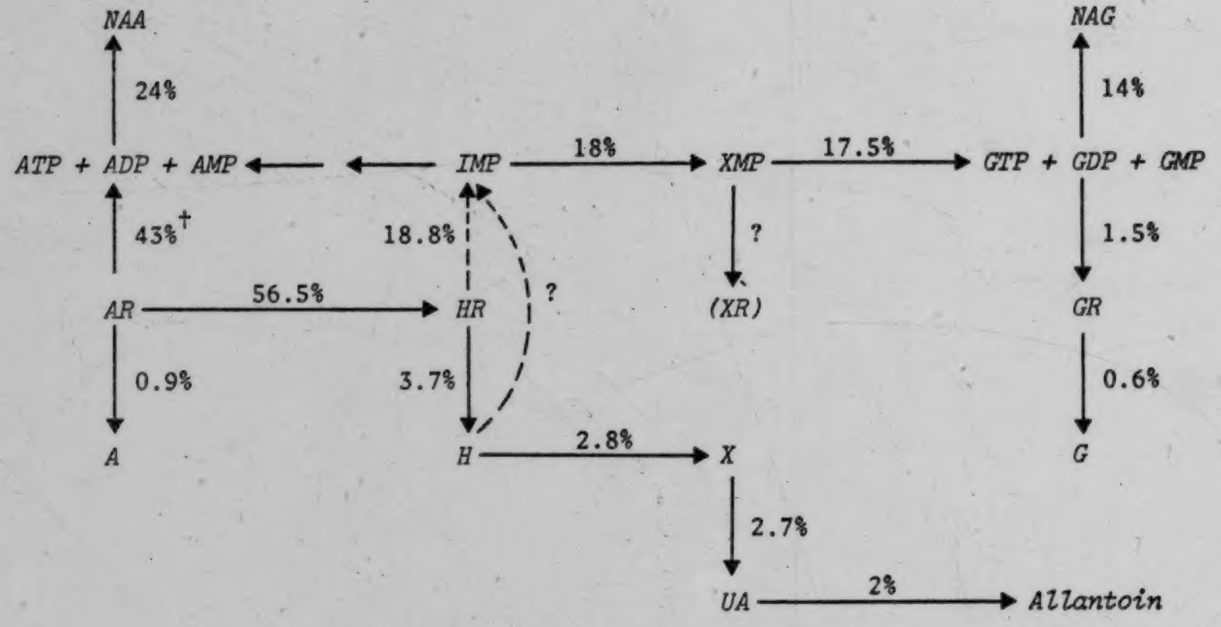
Assuming that deamination of adenosine rather than adenylate is the reaction of major importance in adenosine metabolism, Figure IV-3 shows that most of the adenosine, about 57%, was converted to inosine via adenosine deaminase. The large amount of radioactivity recovered as inosine indicates a high activity of this enzyme.

It is impossible to determine just what fraction of adenine nucleotides was formed from IMP as a result of the activity of adenosine deaminase plus inosine kinase, and what fraction was formed directly from adenosine via adenosine kinase. Accordingly, in the interest of simplicity, no value is placed on the reactions from IMP to adenine nucleotides, although they are shown. It is possible to speculate, however, on the importance of the contribution of these reactions by comparison with the inosine experiments. If it is assumed that the ratio of IMP formation from inosine to catabolism of inosine is roughly the same as when inosine was fed, then about 50% of the radioactivity was converted to IMP in the adenosine experiment. If this is the case, then following the same logic, about 30% of the radioactive adenosine was converted to adenine nucleotides via IMP and only about 13% via adenosine kinase. If this were the case, almost 90% of the adenosine metabolized was deaminated to inosine.

Xanthosine was separated from allantoin in this experiment, but since in other experiments (see Tables IV-3 and IV-6) most of this radioactivity was found to be in the allantoin fraction, this percentage is shown as such in Figure IV-3.

Figure IV-3

Pathways Involved in Adenosine Metabolism



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† Percentages are of the "Total Amount Metabolized" for the Day 2 sample, or 184,147 cpm/μg DNA, which is 80% of the "Total Radioactivity Recovered" (see Table IV-2).

F Metabolism of Radioactive Guanine and Guanosine

It is evident from Table IV-5 that the primary metabolic fate of guanine was degradation. The largest fraction, 52%, was recovered in uric acid, with an additional 32% in xanthine and allantoin. This indicates that guanine deaminase, xanthine dehydrogenase, and uricase are all quite active in the larvae. Since the ratio between the amount of radioactivity in uric acid and allantoin is much higher than in the other experiments, it is probable that uricase activity was saturated under these conditions.

Only negligible amounts of radioactivity were found in nucleic acid and acid-soluble nucleotides, in contrast to the results of the hypoxanthine experiment. Since only one enzyme, hypoxanthine-guanine phosphoribosyltransferase, is generally assumed to be responsible for the utilization of both bases for nucleotide synthesis, this finding is somewhat unexpected. However, in the hypoxanthine experiment, degradation of the precursor was prevented by allopurinol. No equivalent inhibitor of guanine deaminase is available, so that it is not possible to investigate the utilization of guanine in precisely the same way as hypoxanthine. The extensive degradation of guanine may indicate that guanine deaminase has a higher affinity for its substrate than the phosphoribosyltransferase.

Figure IV-4 illustrates the relative importance of catabolic and anabolic pathways when guanine is used as a precursor. It is obvious that most of the guanine was catabolized. Only about 15% was converted to guanine nucleotides, indicating a low activity of guanine phosphoribosyltransferase under these conditions. About 9% of the radioactive guanine nucleotides was converted to IMP and thence to the

Table IV-5

Metabolism of Radioactive Guanine and Guanosine in Wild-Type Larvae[a]

Metabolite	Percent of total amount metabolized	
	Guanine	Guanosine
Nucleic Acid Adenine[b]	0.9	0.0
Nucleic Acid Guanine[b]	0.1	69
AMP + ADP + ATP	0.8	3
GMP + GDP + GTP	0.4	14
IMP	2	0.8
XMP	0.4	1
Adenosine	0.5	1
Inosine	3	0.7
Xanthosine	1	0.9
Guanosine	5	
Succinyl Adenosine	0.4	1
Adenine	0.2	0.3
Hypoxanthine	0.5	0.3
Xanthine	17	3
Guanine		1
Uric Acid	52	3
Allantoin	15	c

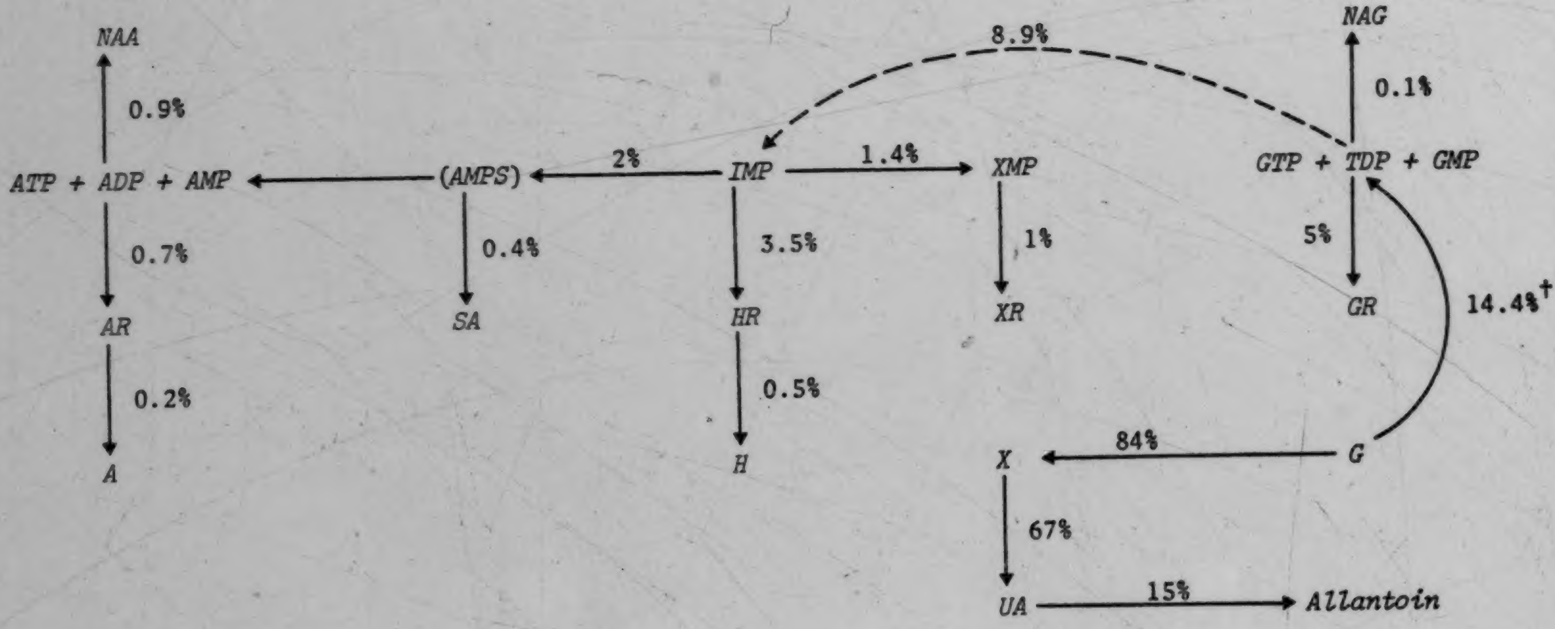
a) Only Day 2 data are shown

b) Nucleic acid adenine/guanine (Guanine experiment) = 7.3

c) Not measured

Figure IV-4

Pathways Involved in Guanine Metabolism



† Percentages are of the "Total Amount Metabolized" for the mean of Day 2 samples, or 60,026 cpm/µg DNA, which is 61% of the "Total Radioactivity Recovered" (see Table IV-2).

other metabolites shown. Presumably this occurred via GMP reductase, although the activity is so low that the line indicating its presence is shown in dashes to imply its relative unimportance.

Adenylosuccinate was not measured in either the guanine or guanosine experiments and so is shown in parentheses in this and the following figures.

Guanosine, by contrast, was primarily anabolized, with 88% of the radioactivity found in nucleic acid and acid-soluble nucleotides. This difference in the utilization of the nucleoside and base again suggests the possibility of nucleoside kinase activity. The arguments for and against the presence of guanosine kinase are basically the same as those for inosine kinase (see discussion of Figure IV-2). Slow conversion to guanine might give the phosphoribosyltransferase a competitive advantage over guanine deaminase (rather than xanthine dehydrogenase) in this case. It cannot be concluded from these results whether guanosine kinase is present or not. It was not found in *Drosophila* cultured cell extracts by Becker (1974b), but Miller and Collins (1973) concluded it was present in *Musca domestica* ovarian extracts.

The fractions of the total radioactivity recovered which were converted to nucleotides were about 33% for the guanosine experiment and about 10% for the guanine experiment. The 33% compares favorably with the 50% found for inosine and adenosine samples.

The most clear-cut conclusion which can be drawn from these results, however, is the fact that *Drosophila* larvae virtually lack GMP reductase activity. No radioactivity was recovered in nucleic acid adenine although 69% was found in nucleic acid guanine, and only

3% was converted to acid-soluble adenine nucleotides in the guanosine experiment. It is difficult to draw any conclusions about this from the guanine experiment due to the negligible amount of guanine which was converted to nucleotides. The low activity of GMP reductase found in this study corroborates the findings of other workers on *Drosophila* cultured cell extracts (Becker, 1974b) and on *Musca domestica* ovarian extracts (Miller and Collins, 1973).

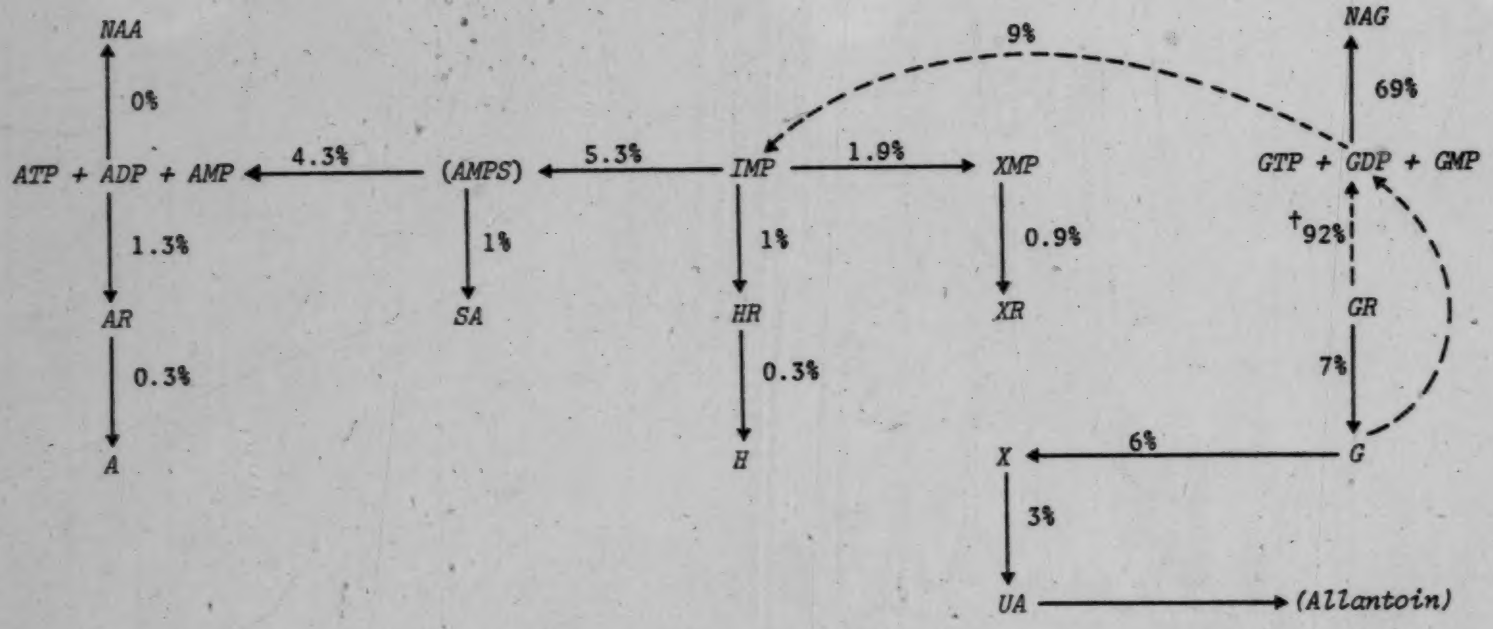
This lack of GMP reductase activity may explain why the fraction of radioactivity recovered which was converted to nucleotides, 33%, was lower than the 50% found in inosine and adenosine samples, and why the "Total Amount Metabolized" fraction was only 38% (see Table IV-2).

Figure IV-5 reflects the uncertainty concerning the presence of guanosine kinase (and GMP reductase) by the use of dashed lines. Allantoin was not measured in this experiment and so is shown in parentheses. Since only 3% of the radioactivity was found in uric acid, it appears unlikely that a significant fraction would have been found in allantoin.

It is interesting to note that the same small fraction of radioactive precursor, 9%, was converted to IMP in both the guanine and guanosine experiments. It must be remembered, however, that in both cases only very small amounts of radioactivity were found in each metabolite resulting from this GMP reductase activity, and the 9% represents the sum of radioactivity in ten different metabolites.

Figure IV-5

Pathways Involved in Guanosine Metabolism



† Percentages are of the "Total Amount Metabolized" for the mean of Day 2 samples, or 58,242 cpm/µg DNA, which is 38% of the "Total Radioactivity Recovered" (see Table IV-2).

G Metabolism of Radioactive Formate

Formate, in these experiments, is assumed to be incorporated into the purine ring in the penultimate step of the *de novo* synthetic pathway as 10-formyl tetrahydrofolate. Aminoimidazolecarboamide was added to the medium to divert available PRPP to the synthesis of its nucleotide, and to reduce the purine synthesis of the earlier segment of the pathway. This ensures that formate fed will be incorporated as the 10-formyl rather than the 5,10-methenyl tetrahydrofolate derivative which serves as a substrate earlier in the pathway. Also, the purine-requiring mutants might not be able to synthesize the aminoimidazolecarboxamide nucleotide themselves.

According to the results shown in Table IV-6, radioactive formate was incorporated quite extensively into purine nucleotides. The two experiments performed show very similar results. About 40% of the radioactivity metabolized was recovered in nucleic acid purines. The ratio of nucleic acid adenine to guanine was the same as the average value of 1.7 found in the hypoxanthine, inosine, and adenosine experiments.

The acid-soluble nucleotide fraction included about 45% of the radioactivity metabolized, and if this fraction is combined with that in nucleic acid, a total of roughly 87% remained as nucleotides. Thus a relatively small amount of the radioactivity in nucleotides was catabolized, the largest fraction in any catabolite being 4.6 or 5% in allantoin. The difference between the two experiments in the nucleoside fractions is probably a result of the different chromatographic techniques used.

Table IV-6

Metabolism of Radioactive Formate in Wild-Type Larvae[a]

Metabolite	Percent of total amount metabolized	
	Exp. 1	Exp. II
Nucleic Acid Adenine[b]	25	25
Nucleic Acid Guanine[b]	15	16
AMP + ADP + ATP	33	39
GMP + GDP + GTP	7	8
IMP	3	1
XMP	1	0.6
AMPS	c	1
Adenosine	0.6	0.2
Inosine	4	0.3
Xanthosine	c	0.07
Guanosine	1	0.3
Adenine	1	0.1
Hypoxanthine	0.25	0.1
Xanthine	0.2	d
Guanine	1	0.5
Uric Acid	2	1
Allantoin	c	4.6
Allantoin + Xanthosine	5	c

a) Only Day 2 data are shown

b) Nucleic acid adenine/guanine: Exp. I = 1.7
Exp. II = 1.6

c) Not measured

d) No measurable radioactivity

Figure IV-6 shows that at the main branch-point of the pathway, IMP, about 60 - 65% of radioactivity recovered was converted to adenine nucleotides and about 25% to guanine nucleotides. Not unexpectedly, this is very close to the percentages obtained in the inosine experiment. About 10% was catabolized.

H Summary and Conclusions

In general, larval DNA content increased from about 0.020 to 0.050 µg from the second to the fourth day in these control experiments, with some variation depending on precursor.

Inosine and adenosine were taken up by the larvae much more efficiently than were the other precursors. Guanine was not very well taken up by larvae. This may have been due either to its insolubility or to increased excretion of this compound.

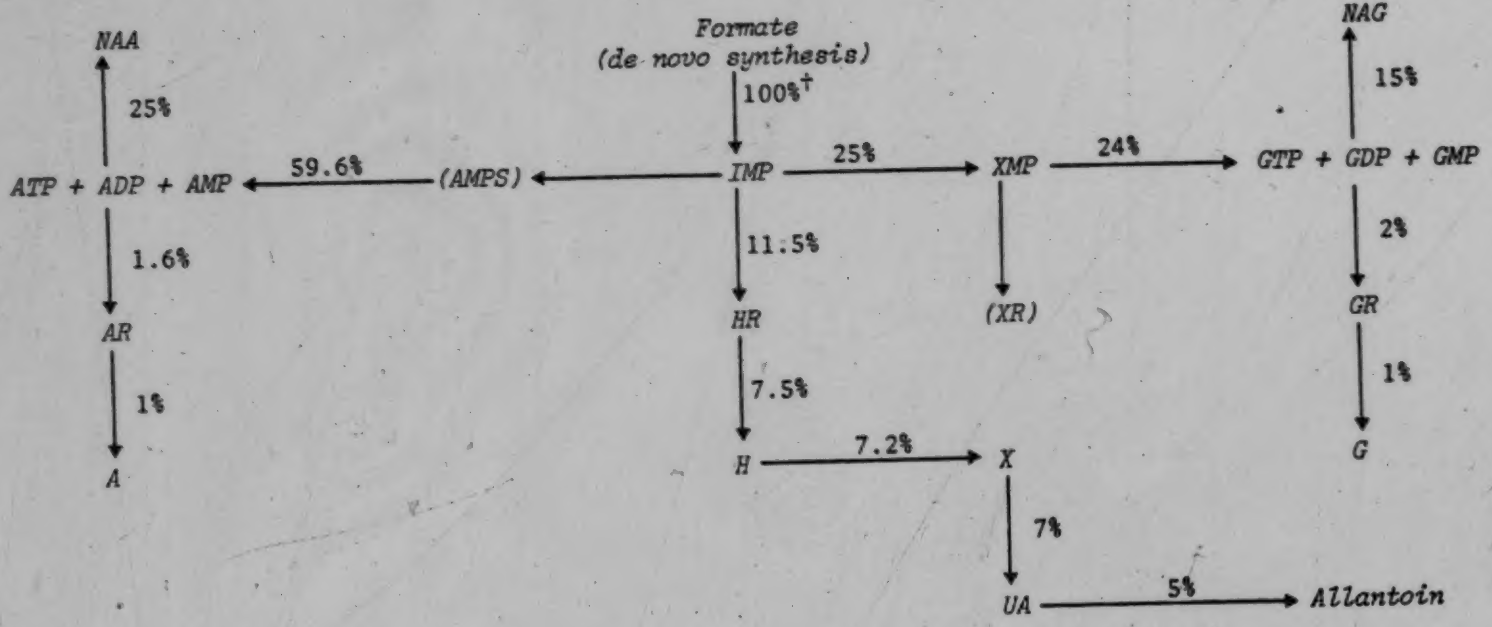
A considerable amount of radioactive hypoxanthine was converted to nucleotides, suggesting activity of hypoxanthine phosphoribosyltransferase. Conflicting reports on this activity from other workers could be explained if only larvae, or perhaps even only certain larval tissues possessed the activity, since other work was done on cultured cells or adult extracts. The very low rate of incorporation into nucleotides observed is consistent with the hypothesis that only one or a few larval tissues possesses the ability to utilize hypoxanthine for nucleotide synthesis.

Evidence has been presented which may indicate the presence of inosine and guanosine kinases.

Figure IV-6

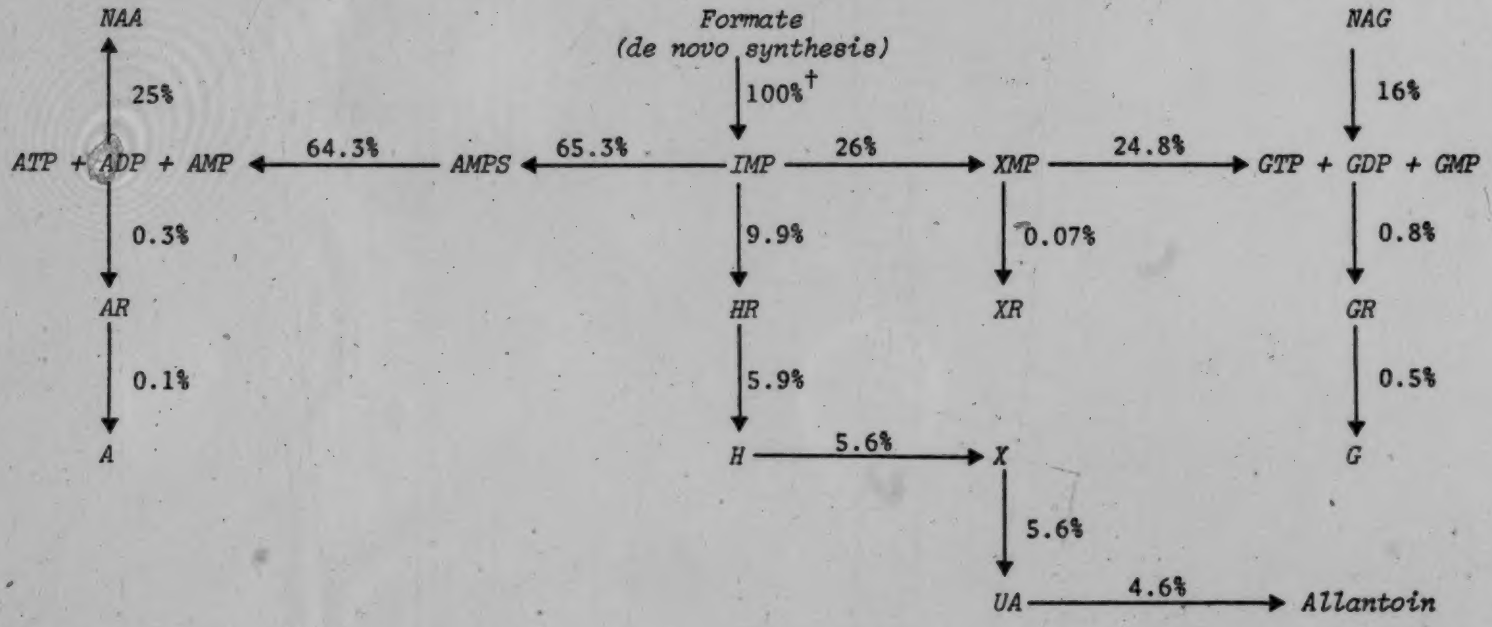
Pathways Involved in Formate Metabolism

Exp I



† Percentages are of the "Total Amount Metabolized" for the mean of Day 2 samples, or 67,795 cpm/µg DNA, which was 44% of the "Total Radioactivity Recovered" (see Table IV-2).

Exp II



† Percentages are of the "Total Amount Metabolized" for the mean of Day 2 samples, or 81,135 cpm/μg DNA, which was 75% of the "Total Radioactivity Recovered" (see Table IV-2).

Extensive conversion of radioactive adenosine to inosine indicates the presence of a very active adenosine deaminase in wild-type larvae.

Guanine deaminase, xanthine dehydrogenase, and uricase were shown to be quite active in *Drosophila* larvae by the extensive degradation of radioactive guanine.

Hypoxanthine-guanine phosphoribosyltransferase appears not to be very active when guanine is a substrate. It may not have as much affinity for guanine as for hypoxanthine in *Drosophila*; or, since no catabolic inhibitor was used in the guanine experiment, the phosphoribosyltransferase might not have as much affinity for its substrate as guanine deaminase.

GMP reductase activity was shown to be almost negligible in both the guanine and the guanosine experiments, confirming other reports of the absence of this enzyme in *Drosophila*.

Radioactive formate with aminoimidazolecarboxamide was incorporated extensively into purine nucleotides.

CHAPTER V BIOCHEMICAL STUDIES OF PURINE-REQUIRING MUTANTS

A Introduction

The purpose of the work presented in this chapter was to investigate the biochemical bases for the genetic defects in several purine-requiring auxotrophs of *Drosophila*. Five such mutants were tested: *pur1-1*, *pur1-2*, *ade1-1*^{sd}, *ade2-1*, and *gua2-1*. The *gua1-1*^{ts} mutant was not tested because no homozygous stock was available. Genetic and nutritional studies on these mutants have been interpreted as suggesting that the *pur1* mutants have a biochemical block in *de novo* purine biosynthesis (Falk and Nash, 1973); that *ade1-1*^{sd} is blocked in AMP synthesis from IMP; and that *gua2-1* is blocked in GMP synthesis from IMP (Johnson, Nash and Henderson, 1977). It has been suggested by Naguib (1976) that the *ade2-1* mutant might have a block in *de novo* purine synthesis, or it might have a mutation in adenylosuccinate lyase (necessary for two steps in the pathway).

The biochemical data obtained for these mutants indicate a much more complicated biochemical situation than the simple hypotheses suggested by the genetic data. One mutant, *ade1-1*^{sd}, showed no large differences from wild type. This is not entirely surprising, since the mutant phenotype is quite weak (it is not lethal but merely slower to develop on defined medium). The data are not presented and the mutant will not be discussed further. The other mutants showed numerous differences from wild type, often apparently unrelated to their mutant phenotype. These differences will be discussed in relation to the original hypothesis for each mutant and an attempt will be made where possible to integrate as many differences as possible into a reasonable alternative hypothesis concerning the cause of the auxotrophy.

However, in general the results were inconclusive for several reasons.

Inevitably in working with higher organisms, there are a number of complications in the interpretation of data such as these. The mutants are not, in a strict sense, comparable to the wild-type controls because they do not grow unless supplemented with high concentrations (3.5 mM) of either adenosine or guanosine. In such circumstances, measurement of specific activity (incorporation per microgram of DNA) might emphasize changes in residual metabolism, while overlooking major effects on net biosynthetic activity. It is not a simple matter to compensate for inherent difficulties in this system. Supplementing concentrations of adenosine or guanosine are, in fact, quite toxic to the wild type, so experiments carried out at any precursor concentration must have a detrimental effect on either the mutants or the wild type. DNA content was used to normalize the amount of radioactivity in each sample in experiments on wild-type and mutant larvae. This may present a problem in the interpretation of the mutant studies since the mutants are ostensibly deficient in purine synthesis and hence, presumably, in DNA synthesis.

Furthermore, experiments such as these do not distinguish which cell type performs the synthesis observed, since the extracts are from whole larvae and represent the sum of purine metabolism in all tissues. It is quite possible that there is a critical tissue which cannot synthesize purines (or a particular purine), or that certain tissues need more purines than can be synthesized at a certain stage of development.

As stated previously, the amount of data generated in a study of this type is very large. Only selected parts of it are presented in this chapter. The rest appears in the Appendix in Tables A-6 through A-27. Tables in this chapter are of the same format as those in the previous chapter, showing a summary of the data presented in the appendix. Specific results which differ substantially from the wild type are underlined.

Also included in this chapter are data from experiments on supplemented wild-type larvae. These experiments were performed to determine the effect of the addition to the medium of supplementing concentrations (3.5 mM) of guanosine on incorporation of radioactive precursors.

B Experimental Conditions and Larval DNA Content

Table V-1 presents the precursors and their concentrations, supplements used, and DNA content per larva in each sample for experiments on mutant larvae. Concentrations of precursors were in most cases the same as those in control experiments, except for the guanosine experiment on the *gua2-1* mutant. In this case the precursor concentration was the same as the usual supplement concentration, 3.5 mM, in order to encourage growth of the mutant as well as to determine the metabolic fate of the supplement. Glycine was not fed to wild-type larvae, but was present in experiments on mutants at a concentration of 1 mM.

It is evident from their DNA content that the mutants, for the most part, did not grow as well as the wild type. Exceptions were *pur1-1* with formate and hypoxanthine, *pur1-2* with formate, and *gua2-1* with guanosine. The mutant larvae generally either showed no increase or only a very small increase in DNA content from the second to the

Table V-1

Experimental Conditions and Larval DNA Content--Mutant *Drosophila*

Mutant	Precursor and Concentration (+ Supplement)	Day and Sample	Number of Larvae/Sample	μg DNA/ Sample	μg DNA/ Larva
pur1-1	Glycine	2a)	90	1.6	0.018
	1 mM	b)	85	1.5	0.018
		4	85	2.0	0.024
	Formate[b]	2a)	90	1.6	0.017
	1 mM	b)	100	3.5	0.035
		4	23	1.1	0.048
	Hypoxanthine[a]	2	90	1.6	0.017
	1 mM	4a)	50	1.5	0.030
		b)	30	1.5	0.050
	Formate[b]	2	90	0.65	0.007
	1 mM	4	24	0.4	0.017
	(+ Guanosine[c])				
pur1-2	Glycine	2	60	1.2	0.020
	1 mM	4	18	0.65	0.036
	Formate[b]	2a)	60	1.6	0.027
	1 mM	b)	95	2.9	0.030
		4	6	0.4	0.067
	Hypoxanthine[a]	2a)	85	1.7	0.020
	1 mM	b)	85	2.1	0.025
		4	60	1.8	0.030
	Formate[b]	2a)	90	1.4	0.016
	1 mM	b)	80	3.0	0.038
	(+ Guanosine[c])	4	15	0.3	0.020

con't.

Table V-1 (con't)

Mutant	Precursor and Concentration (+ Supplement)	Day and Sample	Number of Larvae/Sample	µg DNA/ Sample	µg DNA/ Larva
ade2-1	Glycine	2	120	1.5	0.013
	1 mM	4a)	85	0.8	0.009
		b)	85	1.2	0.014
	Formate[b]	2a)	90	1.9	0.021
	1 mM	b)	60	0.65	0.011
		4	75	2.2	0.029
	Hypoxanthine[a]	2a)	60	0.5	0.008
	1 mM	b)	60	0.65	0.011
		4a)	37	1.0	0.027
		b)	40	0.8	0.020
	Guanine	2a)	90	1.5	0.016
	1 mM	b)	90	1.6	0.018
		4	86	2.1	0.024
	Guanosine	2a)	90	1.2	0.013
	1 mM	b)	90	1.75	0.019
		4a)	50	0.95	0.019
		b)	36	0.7	0.019
	Formate[b]	2a)	90	2.8	0.031
	1 mM	b)	53	1.2	0.023
	(+ Adenosine[c])	4	28	0.6	0.021
gua2-1	Hypoxanthine[a]	2	90	1.35	0.015
	1 mM	4	66	1.2	0.018
	Inosine	2a)	90	1.6	0.018
	0.3 mM	b)	90	1.8	0.020
		4	46	0.9	0.020

con't.

Table V-1 (con't)

Mutant	Precursor and Concentration (+ Supplement)	Day and Sample	Number of Larvae/Sample	μg DNA/ Sample	μg DNA/ Larva
gua2-1	Guanosine	2a)	50	1.2	0.024
	3.5 mM	b)	90	2.7	0.030
		4	10	0.48	0.048
	Inosine	2	85	1.65	0.019
	0.3 mM	4	22	0.4	0.018
	(+ Guanosine[c])				
	Guanine	2	90	2.5	0.028
	1 mM	4	47	1.6	0.034
	(+ Guanosine[c])				

a) 0.5 mM allopurinol added

b) 1 mM aminoimidazolecarboxamide added

c) Supplements were added at a concentration of 3.5 mM fourth days. It is interesting to note that the DNA content on Day 2 is quite similar to the results in control experiments. This result would be expected if the larvae were able to utilize a maternal pool of purines during very early larval life. It is perhaps surprising that the presence of supplements in the medium was not correlated with an increase in DNA content, except for the *gua2-1* mutant.

Table V-2 shows the same information as the previous table for supplemented experiments on wild-type larvae. The fact that these supplements are toxic to the larvae is indicated by the fact that there were so few live larvae left on Day 4 in the two experiments. In general the larval DNA content did not increase as much as in experiments without supplements, probably as a result of the toxicity of these compounds.

C Distribution of Radioactivity Recovered

Table V-3 shows the amount of radioactivity recovered and the amount which was metabolized in counts per minute per microgram of DNA for all the experiments performed on mutant larvae and supplemented wild-type larvae. Day 2 data only are shown in the interest of simplicity. Day 4 data generally showed little change in terms of percentage of recovered radioactivity which was metabolized, in contrast with the increased percentage which was typical of control experiments.

The amount of guanosine recovered in the experiment on *gua2-1* was very large due to the much higher concentration used (3.5 mM). The amount of radioactivity recovered in glycine experiments was also deceptively large in comparison to other precursors due to the high

Table V-2

Experimental Conditions and Larval DNA Content--Supplemented Wild-Type *Drosophila*

Precursor and Concentration	Supplement and Concentration	Day and Sample	Number of Larvae/Sample	μg DNA/Sample	μg DNA/Larva
Formate[a]	Guanosine	2a)	90	2.3	0.025
1 mM	3.5 mM	b)	90	2.0	0.022
		4	55	2.0	0.036
Inosine	Guanosine	2a)	75	1.6	0.021
0.3 mM	3.5 mM	b)	75	2.4	0.032
		4	13	0.6	0.046

a) 1 mM aminoimidazolecarboxamide added

Table V-3

Distribution of Radioactivity Recovered in Mutant and Supplemented Wild-Type Larvae[d]

Strain	Precursor (+ Supplement)	Counts per minute/μg DNA		
		Total Radioactivity Recovered	Unused Precursor	Total Amount Metabolized
pur1-1	Glycine	254,893	154,076	100,817 (40%)[c]
	Formate[b]	136,159	58,670	77,489 (57%)
	Hypoxanthine[a]	84,263	52,524	31,739 (38%)
	Formate[b] (+ Guanosine)	282,292	167,127	115,165 (41%)
pur1-2	Glycine	455,217	318,300	136,917 (30%)
	Formate[b]	96,165	37,979	58,185 (61%)
	Hypoxanthine[a]	127,796	94,704	33,092 (26%)
	Formate[b] (+ Guanosine)	158,805	66,945	91,861 (58%)
ade2-1	Glycine	238,540	163,096	75,444 (32%)
	Formate[b]	89,746	40,602	49,144 (55%)
	Hypoxanthine[a]	269,365	213,083	56,282 (21%)
	Guanine	144,111	110,930	33,181 (23%)
	Guanosine	167,107	36,847	130,261 (78%)
	Formate[b] (+ Adenosine)	97,530	50,409	47,122 (48%)

gua2-1	Hypoxanthine[a]	96,178	64,756	31,422 (33%)
	Inosine	74,427	30,740	43,687 (59%)
	Guanosine	529,251	279,553	249,697 (47%)
	Inosine (+ Guanosine)	89,806	39,652	50,154 (56%)
Wild-Type	Formate[b] (+ Guanosine)	81,003	44,291	36,712 (45%)
	Inosine (+ Guanosine)	89,375	33,696	55,679 (62%)

a) 0.5 mM allopurinol added

b) 1 mM aminoimidazolecarboxamide added

c) Percentages are of the Total Radioactivity Recovered

d) Only Day 2 data are shown, values represent means of replicates where performed (for Day 4 data see Tables A-6, A-11, A-16, and A-22).

specific activity of the glycine used (about twice as high as that of other compounds).

Further details of this table relevant to the auxotrophic phenotype of each mutant will be discussed in the section reserved for that mutant.

Table V-4 shows the percentage of the total amount metabolized which was found in various metabolites of wild-type larvae when supplementary guanosine was added to medium containing radioactive formate and inosine. This table includes data which serve as controls for supplemented experiments on mutants and will be discussed where pertinent.

D Behavior of the *pur1-1* and *pur1-2* Mutants

Four different experiments were performed on each of the two *pur1* mutants, using radioactive glycine, formate, and hypoxanthine. Formate was used both alone and in combination with non-radioactive guanosine as a supplement. The results, shown in Tables V-3, V-5, and V-6, revealed few differences from wild-type; nor were these differences consistent either from precursor to precursor or from one mutant to the other.

Only two findings were compatible with the hypothesis that *pur1-1* is blocked in *de novo* synthesis. First, Table V-5 shows that there was a lower fraction of radioactive formate which was recovered in acid-soluble adenine and guanine nucleotides (for wild type see Table IV-6). This might be expected if the mutant had a biochemical block in one of the last two steps of the pathway of purine synthesis *de novo*. The larvae had only to convert the aminoimidazolecarboxamide present in the

Table V-4

Metabolism of Radioactive Precursors in Supplemented Wild-Type Larvae[a]

	Percent of total amount metabolized	
Metabolite	Formate[b] (+ Guanosine)	Inosine (+ Guanosine)
Nucleic Acid Adenine[d]	39	27
Nucleic Acid Guanine[d]	3	0.7
AMP + ADP + ATP	26	33
GMP + GDP + GTP	5	2
IMP	1	1
XMP	0.5	0.5
Adenine + Adenosine	0.5	0.3
Hypoxanthine + Inosine	1	1[e]
Xanthosine	4	0.3
Guanine + Guanosine	0.9	0.9
Succinyl Adenosine	c	0.3
Xanthine	0.5	6
Uric Acid	8	16
Allantoin	9	9

a) Only Day 2 data shown

b) 1 mM aminoimidazolecarboxamide added

c) Not measured

d) Nucleic acid adenine/guanine: Formate (+ Guanosine): 14.3
Inosine (+ Guanosine): 38.3

e) Precursor excluded

Table V-5

Metabolism of Radioactive Precursors in *pur1-1* Larvae[a]

Metabolite	Percent of total amount metabolized Glycine	 Formate[b]	 Formate[b] (+ Guanosine)	 Hypoxanthine[d]
Nucleic Acid Adenine[f]	49	28	41	19
Nucleic Acid Guanine[f]	7	16	e	17
AMP + ADP + ATP	17	27[h]	37	41
GMP + GDP + GTP	14	4	3	5
IMP	1	1	2	1
XMP	0.6	0.8	0.5	0.5
AMPS	c	c	c	0.5
Adenine + Adenosine	0.1	0.5	0.5	7
Hypoxanthine + Inosine	0.9	0.6	0.8	1[g]
Xanthosine	2	2	0.2	0.4
Guanine + Guanosine	0.9	0.7	0.9	2
Succinyl Adenosine	c	c	c	0.8
Xanthine	e	0.1	1	3
Uric Acid	7	13	9	0.4
Allantoin	1	4	5	1

a) Only Day 2 data shown

b) 1 mM aminoimidazolecarboxamide added

c) Not measured

d) 0.5 mM allopurinol added

e) No measurable radioactivity

f) Nucleic acid adenine/guanine: Glycine: 7.1
Formate: 1.8
Hypoxanthine: 1.1

g) Precursor excluded

h) Underlined numbers represent values which are very different from wild-type.

medium to its nucleotide and complete the last two steps to form IMP. In apparent contradiction to this, the percentage of radioactive formate which was converted to nucleic acid was approximately the same as in the wild type. These two observations are compatible with a modified hypothesis that the larvae have an enzyme deficiency in one of the earlier steps of the *de novo* pathway and use the aminoimidazolecarboxamide (and formate) to supply purines they otherwise could not make. Then, if the concentration of aminoimidazolecarboxamide used, 1 mM, were sub-optimal for supplementation and the purines produced were used preferentially for nucleic acid synthesis, this would explain the deficit in the acid-soluble pool.

The second item of evidence in favor of the original hypothesis is that a greater fraction of radioactivity was incorporated into nucleic acid in the hypoxanthine experiment than in the wild type (see Tables IV-3 and V-5). The nucleic acid adenine fraction was only slightly higher, whereas the nucleic acid guanine fraction was almost twice as high as that found in the wild-type experiment.

Several findings, however, were inconsistent with the hypothesis that the *pur1-1* mutant is deficient in *de novo* purine synthesis. First, it is evident from Tables V-3 and V-5 that these larvae incorporated substantial amounts of radioactive glycine into purines. In addition, Table V-3 shows that only about half as much radioactive hypoxanthine was recovered as in the wild-type experiment (see Table IV-2), although the fraction which was metabolized was about the same. If the mutants lacked the ability to produce IMP, one would expect that any available hypoxanthine would be salvaged at least as efficiently as in the wild-type, if not more so. Finally, the ratio of nucleic acid adenine/guanine in the hypoxanthine experiment (shown at the bottom of Table V-5) was substantially lower than in the wild type (see Table IV-3). This finding is unexpected not only in terms of the original hypothesis but also in view of the fact that this ratio was normal in the formate experiment. Since both precursors are presumed to be converted first to IMP, the reason for this difference was not apparent.

There were several differences from wild type which appeared to be unrelated to the hypothesis being tested. (1) There was an increased percentage of radioactivity metabolized which was found in uric acid and allantoin in the formate experiment (compare Tables IV-6 and V-5). Since this increased catabolism was typical of many experiments on mutants (with the exception of the hypoxanthine experiments, where xanthine dehydrogenase was inhibited) as well as the supplemented experiments on wild type (see Table V-4), this may be a general phenomenon associated with larval death. (2) In the formate plus guanosine experiment the fraction of radioactivity found in acid-soluble adenine nucleotides was 37%, much higher than the 26% found in the control experiment (see Table V-4). The fraction found in acid-soluble guanine nucleotides was somewhat lower than in the wild-type experiment. In nucleic acid purines as well, the adenine fraction was slightly higher and the guanine fraction lower than in the wild type. (3) In an experiment using radioactive glycine with azaserine and supplementary guanosine (data not shown), incorporation of the precursor into FGAR in the *pur1-1* experiment was approximately twice that of the wild type (in cpm/μg DNA), comparable, in fact, to the result obtained from the wild type without guanosine. Considering the fact that guanosine was toxic to the wild type and necessary for the survival of the mutant larvae, the increased fraction in adenine nucleotides and FGAR may be quite understandably due to the different effects on the two strains of the supplement. On the other hand, the reduction in radioactivity recovered in the wild type in the presence of guanosine could be due to feed-back inhibition to which the mutant, presumably with less purines, was apparently not subject. It is difficult to distinguish feed-back inhibition from the effects of dilution and/or reduction in the availability of PRPP due to conversion of guanosine to GMP (if this occurs via hypoxanthine-guanine phosphoribosyltransferase). (4) There was less radioactivity in catabolites in the formate plus guanosine experiment, in the xanthosine and allantoin fractions in particular, possibly associated with better survival in the presence of supplement. (5) The nucleic acid adenine/guanine ratio could not be calculated for the formate plus guanosine experiment since no radioactivity was found in guanine. In the control experiment, shown in Table V-4, the ratio was much higher than in the absence of the supplement. Since a much higher ratio was also obtained in an inosine plus guanosine experiment with wild-type (Table V-4 also), it appears that guanosine has an inhibitory effect on either IMP dehydrogenase or XMP aminase. Snyder and Henderson (1973b) have found that elevated concentrations of GTP have an inhibitory effect on IMP dehydrogenase in intact Ehrlich ascites tumor cells.

At least some of the anomalies in the *pur1-1* data may relate to a secondary hypothesis concerning the mutant; Falk (1973) noted that *pur1-1* (in contrast to *pur1-2*) is better supplemented by guanosine than by adenosine and that, *pur1-1/gua1-1*^{ts} heterozygotes are slow to develop. Both findings suggested a partial block in the conversion of IMP to GMP. Perhaps the simplest interpretation of the present study in light of this suggestion is that the mutant possesses an alteration in one of the two enzymes involved in GMP production from IMP, such that it is both hyperactive as well as more sensitive to inhibition by guanosine. This would account for the lower fractions of radioactivity in nucleic acid guanine and acid-soluble guanine nucleotides in the formate plus guanosine experiment, and for the lower nucleic acid adenine/guanine ratio in the hypoxanthine experiment. Also consistent with this hypothesis is the very low adenine/guanine ratio in the acid-soluble pool in the glycine experiment compared to that found with *pur1-2* and *ade2-1* mutants (Tables V-6 and V-7).

Experiments with the *pur1-2* mutant revealed only two results compatible with the original hypothesis that the larvae were deficient in *de novo* purine synthesis. First, the fraction of radioactivity found in acid-soluble nucleotides in the formate experiment was about half that found in the wild-type experiment (compare Tables IV-6 and V-6). Also, the nucleic acid adenine fraction was 35% for the mutant, compared to only 25% in the wild type. These findings are similar to those in the *pur1-1* formate experiment and the same discussion applies to *pur1-2*, with the exception of the increased nucleic acid adenine fraction. The other result compatible with the original hypothesis was the increased fraction of radioactivity in both nucleic acid purines compared to the wild-type in the hypoxanthine experiment (see Tables IV-3 and V-6).

Table V-6

Metabolism of Radioactive Precursors in *pur1-2* Larvae[a]

	Percent of total amount metabolized			
Metabolits	Glycine	Formate[b]	Formate[b] (+ Guanosine)	Hypoxanthine[c]
Nucleic Acid Adenine[f]	57	35[h]	34	24
Nucleic Acid Guanine[f]	9	19	2	16
AMP + ADP + ATP	13	17	28	41
GMP + GDP + GTP	7	4	4	7
IMP	0.9	2	6	2
XMP	0.3	0.7	1	0.3
AMPS	0.1	d	d	d
Adenine + Adenosine	0.2	0.4	0.7	3
Hypoxanthine + Inosine	0.8	0.8	0.6	1[g]
Xanthosine	3	2	3	0.3
Guanine + Guanosine	5	1	0.5	0.7
Succinyl Adenoisne	d	d	d	1
Xanthine	e	0.2	0.2	3
Uric Acid	2	4	5	1
Allantoin	1	12	14	0.1

a) Only Day 2 data shown

b) 1 mM aminoimidazolecarboxamide added

c) 0.5 mM allopurinol added

d) Not measured

e) No measurable radioactivity

f) Nucleic acid adenine/guanine: Glycine: 6.6
Formate: 1.9
Hypoxanthine: 1.5
Formate (+ Guanosine): 17.5

g) Precursor excluded

h) Underlined numbers represent values which are very different from wild-type.

Findings which were inconsistent with the original hypothesis were: (1) the substantial incorporation of radioactive glycine (see Table V-3), and (2) the high percentage of radioactivity in IMP in the formate plus guanosine experiment (see Table V-6).

These experiments showed two results which appeared unrelated to the hypothesis being tested, these being: (1) the increased catabolism characteristic of the formate experiments (see Table V-6), and (2) the increased amount of radioactive formate which was both recovered and metabolized in the experiment supplemented with guanosine (see Table V-3). As in the case of the *pur1-1* mutant, this latter difference from the wild type may be a result either of the detrimental effect of the supplement on wild type or of its beneficial effect on the mutant in terms of growth.

The two *pur1* mutants are allelic and hence might be expected to produce similar results in these experiments. However, there were several differences: (1) the amount of glycine incorporation into FGAR in the presence of guanosine and azaserine was similar to the wild-type value rather than elevated as in *pur1-1* (data not shown); (2) in both formate experiments with *pur1-2*, the allantoin fraction was much higher and the uric acid fraction much lower than in *pur1-1* (Tables V-5 and V-6). Other differences in adenine/guanine ratios were probably due to the previously mentioned differences with respect to supplementation and complementation.

The basic cause of the auxotrophy of these mutants was not revealed by these techniques, although it is apparent that there is some difference between the two in the metabolism of guanine nucleotides. There was some evidence for an alteration of function of either

IMP dehydrogenase or XMP aminase in *pur1-1*, but not in *pur1-2*.

E Behavior of the *ade2-1* Mutant

The *ade2-1* mutant was isolated by Naguib (1976) who found that it would supplement either with adenosine or with inosine. She described three possible explanations for its behavior: (1) a defect in one of the enzymes of *de novo* synthesis; (2) a defect in AMPS lyase, needed for both *de novo* synthesis and synthesis of AMP from IMP; or (3) two distinct mutations, since mapping data were ambiguous.

There was very little evidence in the present study to support any of these hypotheses. In the guanine experiment the nucleic acid adenine fraction was lower and the nucleic acid guanine fraction higher than in the control experiment (Tables IV-5 and V-7). In the guanosine experiment, the acid-soluble adenine nucleotide fraction was lower than in wild type. The results are consistent with, but not supportive of the hypothesis, since they are presumably dependent on GMP reductase activity which was very low in wild type.

The majority of the results shown in Table V-7 could be explained if the mutation resulted in a reduced activity of guanine deaminase. (1) In the formate experiment, a high fraction of radioactivity was found in the guanine plus guanosine fraction (compare with Table IV-6). (2) In the guanine experiment, the fraction in uric acid was much lower than in the wild type, and the guanosine and allantoin fractions were higher (Tables IV-5 and V-7). (3) A relatively large percentage of the amount metabolized in the latter experiment was found in guanine (Tables IV-5 and V-7). (4) There was a much lower fraction of guanine which was metabolized (Tables IV-2 and V-3).

Table V-7

Metabolism of Radioactive Precursors in *ade3-1* Larvae[a]

Metabolite	Percent of total amount metabolized					
	Glycine	Formate[b]	Formate[b] (+ Adenosine)	Guanine	Guanosine	Hypoxanthine[c]
Nucleic Acid Adenine[e]	40	24	17	$\underline{0.2}$	d	18
Nucleic Acid Guanine[e]	1	15	6	$\underline{3}$	$\underline{53}$	8
AMP + ADP + ATP	32	31	21	1	$\underline{0.7}$	44
GMP + GDP + GTP	7	8	5	$\underline{2}$	12	6
IMP	2	1	1	1	0.5	2
XMP	1	0.6	0.5	0.7	0.3	0.3
AMPS	0.9	0.7	0.5	0.2	0.1	0.6
Adenine + Adenosine	0.3	0.4	0.5	1	1	5
Hypoxanthine + Inosine	1	1	6	$\underline{14}$	1	2^f
Xanthosine	0.3	2	0.4	1	0.3	0.2
Guanine + Guanosine	2	$\underline{10}^g$	23	$\underline{13}^f$	$\underline{19}^f$	2
Succinyl Adenosine	d	d	d	2	0.2	2
Xanthine	0.1	0.04	0.3	13	4	7
Uric Acid	5	1	9	$\underline{14}$	3	0.8
Allantoin	6	4	10	$\underline{37}$	5	0.9

a) Only Day 2 data shown

b) 1 mM aminoimidazolecarboxamide added

c) 0.5 mM allopurinol added

d) Not measured

e) Nucleic acid adenine/guanine: Glycine: 27.1; Formate: 1.6; Hypoxanthine: 1.9; Guanine: 0.13;
Formate (+ Adenosine): 2.9

f) Precursor excluded

g) Underlined numbers represent values which are very different from wild-type.

(5) If the reduced activity were to give guanine phosphoribosyltransferase a competitive advantage, the increased fraction on incorporation of radioactive guanine into nucleic acid and acid-soluble nucleotides would be explained.

Wyss (1977) has shown that toxicity of guanosine to cultured *Drosophila* cells can be counteracted by adenine, adenosine, or inosine. If the proposed reduction in guanine deaminase resulted in toxic concentrations of either guanosine, or more likely guanine nucleotides, Wyss' finding would neatly explain the auxotrophic phenotype (Naguib did not test adenine). It would also explain a number of findings which were inconsistent with her hypotheses. (1) There was a substantial amount of glycine found in purines shown in Table V-3. (2) The nucleic acid adenine/guanine ratio in the glycine experiment was even higher than for the *pur1* mutants (Tables V-5, V-6, V-7). This makes it appear that *ade2-1* makes more rather than less adenine nucleotides relative to guanine nucleotides, which could conceivably represent an attempt on the part of the organism itself to counteract the increase in guanine nucleotides (probably via the inhibitory effect of these compounds on IMP dehydrogenase; see Snyder and Henderson, 1973b). However, since the reason for the elevated ratios in the *pur1* mutants is unknown, this even greater elevation has to be considered enigmatic. (3) The similarity of the results of the formate experiment to the wild type (compare Tables IV-6 and V-7), with the exception of the guanine plus guanosine fraction, also seems to indicate that the mutant has no difficulty in synthesizing purines. The quite normal nucleic acid adenine/guanine ration in this experiment casts further doubt on the significance of the elevated ratio in the glycine experiment and the reduced ratios in the guanine and guanosine experiments. (4) In the formate plus adenosine experiment, the increased nucleic acid adenine/guanine ratio is surprising in terms of Naguib's original hypotheses. In wild-type experiments, this ratio was similar with both formate and inosine as precursors; when inosine was supplemented with adenosine (data not shown), the ratio was reduced to about 1.3. If IMP dehydrogenase (or XMP aminase) were inhibited by the higher guanine nucleotide concentrations, this relative increase would be understandable.

Inconsistent with the hypothesis proposed here were the findings that (1) the nucleic acid adenine/guanine ratio in the formate experiment was normal, and that (2) the fractions of radioactive hypoxanthine found in all metabolites were similar to the wild type.

F Behavior of the *gua2-1* Mutant

The *gua2-1* mutant was isolated by Nash (Johnson, Nash and Henderson, 1977), who found that it could be supplemented only with guanosine. His theory that the mutant is defective in one of the two enzymes which produce GMP from IMP received some support from the data presented in Table V-8.

In both the hypoxanthine and the inosine experiments, the adenine/guanine ratio in acid-soluble nucleotides was greater than in the wild type (Table IV-3). In the hypoxanthine and the inosine plus guanosine experiments, a higher fraction of radioactivity was found in adenine plus adenosine than in the wild type (Tables IV-3 and V-2). Also consistent with the original theory was the very large nucleic acid adenine/guanine ratio in the inosine plus guanosine experiment.

Table V-8

Metabolism of Radioactive Precursors in *gua2-1* Larvae[a]

Metabolite	Percent of total amount metabolized				
	Hypoxanthine[b]	Inosine	Inosine (+ Guanosine)	Guanosine	Guanine (+ Guanosine)
Nucleic Acid Adenine[d]	21[f]	24	27	c	0.2
Nucleic Acid Guanine[d]	13	13	c	33	0.2
AMP + ADP + ATP	30	23	28	3	2
GMP + ADP + ATP	3	2	1	13	0.4
IMP	1	2	1	0.9	0.8
XMP	0.4	0.5	0.5	1	0.3
Adenine + Adenosine	15	0.3	2	1	0.9
Hypoxanthine + Inosine	1[e]	1[e]	0.6[e]	1	4
Xanthosine	0.4	0.4	0.5	0.9	7
Guanine + Guanosine	2	0.8	2	0.6[e]	4[e]
Xanthine	12	0.6	2	3	7
Uric Acid	0.1	30	22	10	58
Allantoin	0.1	2	3	31	22

a) Only Day 2 data shown

b) 1 mM allopurinol added

c) No measurable radioactivity

d) Nucleic acid adenine/guanine: Hypoxanthine: 1.5; Inosine: 1.8; Inosine (+ Guanosine): 72.7; Guanine (+ Guanosine): 1.0

e) Precursor excluded

f) Underlined numbers represent values which are very different from wild-type.

If, as was previously speculated, guanosine has an inhibitory effect on one of the two enzymes involved, it is not inconceivable that the enzyme affected might be the one which also possessed a reduced activity in the mutant, causing the much higher adenine/guanine ratio in the mutant.

Possibly inconsistent with the hypothesis was the finding that the nucleic acid adenine/guanine ratios in the hypoxanthine and inosine experiments were approximately the same as the wild type (Table IV-3).

The increased catabolism in the hypoxanthine, inosine, and guanosine experiments may be correlated with the generally unhealthy phenotype of mutant larvae.

G Conclusions

Evidence was presented which suggests that the *ade2-1* mutant may have a reduced activity of guanine deaminase. In general, however, there was very little that could be concluded definitely about the results of the biochemical studies performed on the mutants, except that the cause of the auxotrophic phenotype in *Drosophila* appears to be much more complex than that involved in auxotrophic counterparts in microbes or in isolated cell lines. Tissue specialization is a key factor in development and evolution of such an organism; thus it appears most likely that tissue differences either in terms of production or utilization of purines are responsible for the failure to detect clear-cut, consistent differences which would define the cause of the auxotrophy.

A more fruitful means of investigating the bases of the auxotrophy of the *pur1* mutants, therefore, might be the culture of individual tissues and/or organs, using similar radioactive tracer techniques to determine blocked pathways. In the case of *ade2-1*, an assay of guanine deaminase would provide important evidence either for or against the hypothesis presented here. Work is proceding in Nash's laboratory to test IMP dehydrogenase activity in the *gua2-1* mutant, with thus far promising results (Nash, personal communication).

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APPENDIX

Table A-1

Catabolism of Precursors in Wild-Type Larvae

Precursor (+ Supplement)	Day and Sample	Total Amount Catabolized	Counts per minute/µg DNA						
			Adenine	Hypoxanthine	Inosine	Guanine	Xanthine	Uric Acid	Allantoin
Adenosine	2	70,506 (38%)[b]	1,731 (0.9%)	1,769 (0.9%)	62,181 (34%)	-[e]	256 (0.1%)	1,287 (0.7%)	3,281[c] (2%)
	4a)	18,021	1,226	1,879	5,741	-	1,507	1,275	6,391[c]
	b)	23,708	479	2,394	9,606	-	479	1,089	9,610[c]
		20,865[d] (16%)	853 (0.7%)	2,137 (2%)	7,673 (6%)		993 (0.7%)	1,182 (0.9%)	8,001[c] (6%)
Hypoxanthine[a]	2a)	2,028	-	-	-	-	1,550	405	72
	b)	2,340	-	-	-	-	2,220	20	100
		2,184 (7%)					1,885 (5%)	213 (0.6%)	86 (0.2%)
	4a)	5,786	-	-	-	-	2,237	3,247	302
	b)	2,940	-	-	-	-	2,600	28	312
		4,363 (5%)					2,419 (3%)	1,637 (2%)	307 (0.3%)
Inosine Exp. I	2a)	5,067	-	2,311	-	-	778	317	1,661[c]
	b)	5,700	-	2,539	-	-	477	561	2,122[c]
		5,383 (6%)		2,425 (3%)			627 (0.8%)	439 (0.5%)	1,891[c] (2%)
	4a)	9,428	-	3,304	-	-	1,075	740	4,309[c]
	b)	9,637	-	3,181	-	-	250	675	5,531[c]
	c)	8,311	-	2,932	-	-	278	521	4,579[c]
		9,124 (5%)		3,139 (2%)			534 (0.3%)	645 (0.3%)	4,806[c] (3%)

Inosine	2	4,568 (8%)	-	1,123 (2%)	-	-	427 (0.7%)	1,195 (2%)	1,823 (3%)
Exp. II	4	17,587 (13%)	-	1,500 (1%)	-	-	731 (0.5%)	6,025 (4%)	9,331 (7%)
Guanine	2a)	54,400	-	-	-	-	11,738	33,469	9,193
	b)	47,294	-	-	-	-	9,171	29,288	8,835
		50,847 (84%)					10,455 (17%)	31,379 (52%)	9,014 (15%)
	4	24,929 (74%)	-	-	-	-	12,686 (38%)	5,218 (15%)	7,025 (21%)
Guanosine	2a)	2,834	-	-	-	556	1,696	581	g
	b)[f]	6,323	-	-	-	621	3,347	2,354	g
	c)	2,935	-	-	-	745	710	1,480	g
		4,031 (7%)				641 (1%)	1,918 (3%)	1,472 (3%)	
	4	6,849 (9%)	-	-	-	2,753 (3%)	3,133 (4%)	983 (1%)	g
Inosine	2a)	7,280	-	615	-	-	280	1,165	5,220
(+ Adenosine)	b)	7,896	-	365	-	-	407	2,152	4,972
		7,588 (18%)		490 (1%)			343 (0.8%)	1,659 (4%)	5,096 (12%)
	4 [f]	10,932 (31%)	-	3,098 (9%)	-	-	1,484 (4%)	1,435 (4%)	4,915 (14%)
Inosine	2a)	15,063	-	513	-	-	3,375	7,500	3,675
(+ Guanosine)	b)	9,111	-	320	-	-	1,104	4,608	3,079
		12,087 (33%)		417 (1%)			2,239 (6%)	6,054 (16%)	3,377 (9%)
	4	26,817 (18%)	-	8,417 (6%)	-	-	700 (0.5%)	3,733 (3%)	13,967 (10%)

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a) 0.5 mM allopurinol added

b) Percentages of Total Amount Metabolized (from Tables IV-2 and V-3)

c) Values include a small number of counts in xanthosine

d) Underlined numbers are the means of two or three preceding samples

e) Dashes indicate compounds which are not considered precursor catabolites of a particular precursor

f) All larvae in this sample were dead

g) Not measured

Table A-2

Metabolism of Radioactive Nucleotides in Wild-Type Larvae

Precursor (+ Supplement)	Day and Sample	Counts per minute/µg DNA Total Conversion to Nucleotides	 Amount in Nucleic Acids	 Amount in Acid-Soluble Nucleotides	 Amount Catabolized
Adenosine	2	113,641 (62%)^{c}	69,554 (38%)	41,337 (22%)	2,750^{d} (1%)
	4a)	106,840	64,259	40,502	2,079^{d}
	b)	105,369	56,167	48,265	937^{d}
		106,103^{e} (84%)	60,213 (47%)	44,383 (35%)	1,508^{d} (1%)
Hypoxanthine^{a}	2a)	31,510	9,499	18,027	3,984
	b)	34,046	9,153	21,440	3,453
		32,779 (94%)	9,326 (26%)	19,734 (56%)	3,719 (11%)
	4a)	93,120	37,160	50,162	5,798
	b)	73,854	26,172	40,980	6,702
		83,488 (95%)	31,666 (37%)	45,572 (52%)	6,250 (5%)
Inosine	2a)	67,849	28,099	37,700	2,050^{d}
Exp. I	b)	85,993	38,632	45,122	2,239^{d}
		76,921 (94%)	33,365 (41%)	41,411 (50%)	2,145^{d} (3%)
	4a)	160,418	103,690	49,729	6,999^{d}
	b)	177,012	99,752	70,678	6,582^{d}

	c)	179,702		95,019		76,207		8,476[d]	
		172,377	(95%)	99,487	(55%)	65,538	(36%)	7,352[d]	(4%)
Inosine	2	54,261	(92%)	31,327	(53%)	22,353	(38%)	581	(1%)
Exp. II	4	117,154	(87%)	58,916	(40%)	60,169	(45%)	3,069	(2%)
Guanine	2a)	11,365		821		2,572		7,972	
	b)	6,993		429		1,764		4,800	
		9,179	(16%)	625	(1%)	2,168	(4%)	6,386	(11%)
		8,960	(26%)	1,160	(3%)	1,614	(5%)	6,186	(18%)
Guanosine	2a)	42,270		32,744		8,152		1,875	
	b)[f]	68,320		50,524		14,891		2,905	
	c)	51,543		38,028		10,730		2,785	
		54,211	(93%)	40,432	(70%)	11,258	(19%)	2,522	(4%)
	4	68,483	(91%)	58,406	(78%)	2,308	(3%)	7,769	(10%)
Formate[b]	2a)	68,920		26,715		28,910		13,295	
Exp. I	b)	66,669		27,353		29,783		9,533	
		67,794	(100%)	27,034	(40%)	29,247	(43%)	11,414	(17%)
	4	108,699	(100%)	72,028	(66%)	26,864	(25%)	9,807	(9%)
Formate[b]	2a)	60,059		31,037		23,061		5,961	
Exp. II	b)	102,212		36,801		59,326		6,085	
		81,135	(100%)	33,919	(42%)	41,193	(51%)	6,023	(7%)

con't.

Table A-2 (cont'd)

Precursor (+ Supplement)	Day and Sample	Counts per minute/μg DNA Total Conversion to Nucleotides	Amount in Nucleic Acids	Amount in Acid Soluble Nucleotides	Amount Catabolized
	4a)	84,642	52,203	25,144	7,295
	b)	79,121	47,441	24,820	6,860
		81,882 (100%)	49,822 (61%)	24,982 (31%)	7,077 (8%)
Formate[b]	2a)	47,925	21,633	15,074	11,218
(+ Guanosine)	b)	44,302	17,187	15,760	11,355
		46,113 (100%)	19,410 (42%)	15,417 (33%)	11,287 (24%)
	4	82,611 (100%)	32,271 (39%)	31,920 (39%)	18,420 (22%)
Inosine	2a)	29,087	14,397	13,455	1,235
(+ Adenosine)	b)	38,594	21,891	14,952	1,752
		33,841 (82%)	18,144 (44%)	14,203 (34%)	1,493 (4%)
	4[f]	24,348 (69%)	8,881 (25%)	12,004 (34%)	3,463 (10%)
Inosine	2a)	26,107	9,507	15,875	725
(+ Guanosine)	b)	23,523	10,731	11,833	578
		24,815 (67%)	10,119 (27%)	13,854 (37%)	652 (2%)
	4	117,659 (81%)	61,518 (43%)	52,558 (36%)	3,583 (2%)

a) 0.5 mM allopurinol added

b) 1 mM aminoimidazolecarboxamide added

c) Percentages of Total Amount Metabolized (from Tables IV-2 and V-)

d) Excluding a small number of counts in xanthosine

e) Underlined numbers are the means of two or three preceding samples

f) All larvae in this sample were dead

Table A-3

Distribution of Radioactivity in Acid-Soluble Nucleotides in Wild-Type Larvae

Precursor (+ Supplement)	Day and Sample	Counts per minute/µg DNA						Adenine/ Guanine Ratio[b]
		Remained as Acid-Soluble Nucleotides	Adenine Nucleotides	Guanine Nucleotides	IMP	AMPS	XMP	
Adenosine	2	41,337 (22%)[c]	34,344 (19%)	4,619 (2%)	1,519 (0.8%)	d	856 (0.5%)	7.4
	4a)	40,502	30,396	5,366	3,560	d	1,179	5.7
	b)	48,265	36,192	7,023	3,454	d	1,596	5.1
		44,383[e] (35%)	33,294 (26%)	6,195 (5%)	3,507 (3%)		1,387 (1%)	5.4
Hypoxanthine[a]	2a)	18,027	14,661	1,861	722	505	278	8.1
	b)	21,440	17,440	2,180	847	620	353	8.2
		19,734 (56%)	16,050 (46%)	2,021 (6%)	785 (2%)	563 (2%)	315 (0.9%)	8.1
	4a)	50,162	38,116	7,405	2,023	1,837	781	5.4
	b)	40,980	32,336	4,752	1,616	1,416	860	7.1
		45,572 (52%)	35,226 (40%)	6,079 (7%)	1,819 (2%)	1,627 (2%)	821 (0.9%)	6.3
Inosine	2a)	37,700	27,283	6,867	2,233	d	1,317	4.0
	b)	45,122	33,478	7,711	2,672	d	1,261	4.3
		41,411 (50%)	30,381 (37%)	7,289 (9%)	2,437 (3%)		1,289 (1%)	4.1
	4a)	49,729	35,309	7,253	4,635	d	2,532	4.9
	b)	70,678	46,250	12,734	6,856	d	4,838	3.6
	c)	76,207	50,638	14,303	7,198	d	4,068	3.5
		65,538 (36%)	44,066 (24%)	11,430 (6%)	6,230 (3%)		3,812 (2%)	4.0
Inosine	2	22,353 (38%)	17,772 (30%)	2,527 (4%)	1,250 (2%)	459 (0.7%)	345 (0.6%)	7.2
Exp II	4	60,169 (45%)	49,825 (37%)	6,656 (5%)	2,163 (2%)	731 (0.5%)	794 (0.5%)	7.6
Guanine	2a)	2,572	600	283	1,386	d	303	2.1
	b)	1,764	406	176	947	d	206	2.3
		2,168 (3%)	503 (0.8%)	229 (0.4%)	1,167 (2%)		255 (0.4%)	2.2
	4	1,614 (5%)	384 (1%)	170 (0.5%)	864 (3%)	d	195 (0.6%)	2.3
Guanosine	2a)	8,152	1,349	5,966	354	d	462	0.23
	b)[f]	14,891	2,495	10,617	754	d	1,025	0.23
	c)	10,730	1,810	8,050	290	d	580	0.22
		11,258 (19%)	1,885 (3%)	8,211 (14%)	466 (0.8%)		696 (1%)	0.23
	4	2,308 (3%)	446 (0.6%)	861 (1%)	553 (0.5%)	d	446 (0.6%)	0.52

Table A-3 (con't)

Precursor (+ Supplement)	Day and Sample	Counts per minute/ug DNA						Adenine/ Guanine Ratio
		Remained as Acid-Soluble Nucleotides	Adenine Nucleotides	Guanine Nucleotides	IMP	AMPS	XMP	
Formate[b]	2a)	28,910	21,830	4,595	1,815	d	670	4.7
Exp I	b)	29,783	22,617	4,611	1,689	d	867	4.9
		29,247 (43%)	22,223 (33%)	4,603 (7%)	1,752 (3%)		769 (1%)	4.8
	4	26,864 (25%)	19,652 (18%)	4,379 (4%)	1,923 (2%)	d	909 (0.8%)	4.5
Formate[b]	2a)	23,061	17,322	4,294	644	450	350	4.1
Exp II	b)	59,326	45,937	9,053	2,231	1,463	642	5.2
		41,193 (50%)	31,629 (39%)	6,673 (8%)	1,437 (1%)	957 (1%)	496 (0.6%)	4.7
	4a)	25,144	18,245	4,328	1,200	844 (1%)	528	4.4
	b)	24,820	18,567	4,213	1,527	d	513	4.4
		24,982 (30%)	18,406 (23%)	4,271 (5%)	1,363 (1%)		521 (0.6%)	4.4
Formate[b]	2a)	15,074	11,630	2,513	643	d	287	4.6
(+ Guanosine)	b)	15,760	12,795	2,135	640	d	190	6.0
		15,417 (33%)	12,212 (26%)	2,324 (5%)	641 (1%)		239 (0.5%)	5.3
	4	31,920 (39%)	20,660 (25%)	10,320 (12%)	615 (0.7%)	d	325 (0.4%)	2.0
Inosine	2a)	13,455	11,070	1,505	600	d	280	7.4
(+ Adenosine)	b)	14,952	12,434	1,883	483	d	152	6.6
		14,203 (34%)	11,752 (28%)	1,694 (4%)	541 (1%)		216 (0.5%)	7.0
	4[f]	12,004 (34%)	8,569 (24%)	1,693 (5%)	1,560 (4%)	d	182 (0.5%)	5.1
Inosine	2a)	15,875	14,081	919	669	d	206	15.3
(+ Guanosine)	b)	11,833	10,675	600	404	d	154	17.8
		13,854 (37%)	12,378 (33%)	759 (2%)	537 (1%)		180 (0.5%)	16.5
	4	52,558 (36%)	45,408 (31%)	4,850 (3%)	3,717 (3%)	d	883 (0.6%)	9.4

a) 0.5 mM allopurinol added

b) 1 mM aminoimidazolecarboxamide added

c) Percentages of Total Amount Metabolized (from Tables IV-2 and V-3)

d) Not measured

e) Underlined numbers are the means of two or three preceding samples

f) All larvae in this sample were dead

Table A-4

Incorporation of Precursor into Nucleic Acid by Wild-Type Larvae

Precursor (+ Supplement)	Day and Sample	Counts per minute/ug DNA						Adenine/Guanine Ratio
		Total Incorporation into Nucleic Acid		Nucleic Acid Adenine		Nucleic Acid Guanine		
Adenosine	2	69,554	(38%)[c]	43,377	(24%)	26,177	(14%)	1.7
	4a)	64,259		38,887		25,372		1.5
	b)	56,167		33,873		22,294		1.5
		60,213[d]	(47%)	36,380	(28%)	23,833	(19%)	1.5
Hypoxanthine[a]	2a)	9,499		5,982		3,517		1.7
	b)	9,153		5,619		3,534		1.6
		9,326	(26%)	5,801	(16%)	3,525	(10%)	1.7
	4a)	37,160		22,011		15,149		1.5
	b)	26,172		15,889		10,283		1.5
		31,660	(37%)	18,950	(22%)	12,716	(15%)	1.5
Inosine	2a)	28,099		19,463		8,636		2.2
Exp. I	b)	38,632		24,569		14,063		1.7
		33,365	(41%)	22,016	(27%)	11,349	(14%)	1.9
	4a)	103,690		67,714		35,976		1.9
	b)	99,752		60,732		39,020		1.5
	c)	95,019		61,576		33,443		1.8
		99,487	(55%)	63,341	(35%)	36,146	(20%)	1.7

Inosine	2	31,327 (53%)	18,991 (32%)	12,336 (21%)	1.5
Exp. II	4	53,916 (40%)	31,889 (24%)	22,027 (16%)	1.4
Guanine	2a)	821	711	110	6.5
	b)	429	382	47	8.1
		625 (1%)	547 (0.9%)	79 (0.1%)	7.3
	4	1,160 (3%)	715 (2%)	445 (1%)	1.6
Guanosine	2a)	32,744	e	32,744	
	b)^f	50,524	e	50,524	
	c)	38,028	e	38,028	
		40,432 (69%)		40,432 (69%)	
	4	58,406 (81%)	e	58,406 (81%)	
Formate^b	2a)	26,715	16,645	10,070	1.7
Exp. I	b)	27,353	17,170	10,183	1.7
		27,034 (40%)	16,907 (25%)	10,127 (15%)	1.7
	4	72,028 (66%)	45,907 (42%)	26,121 (24%)	1.7
Formate^b	2a)	31,037	19,013	12,024	1.6
Exp. II	b)	36,801	22,684	14,117	1.6
		33,919 (41%)	20,849 (25%)	13,071 (16%)	1.6
	4a)	52,203	35,147	17,056	2.1
	b)	47,441	31,854	15,587	2.0
		49,822 (60%)	33,501 (40%)	16,321 (20%)	2.1

con't

Table A-4 (con't)

Precursor (+ Supplement)	Day and Sample	Counts per minute/µg DNA Total Incorporation into Nucleic Acid		Nucleic Acid Adenine		Nucleic Acid Guanine		Adenine/Guanine Ratio
Formate[b]	2a)	21,633		19,609		2,024		9.7
(+ Guanosine)	b)	17,187		16,324		863		18.9
		19,410	(42%)	17,967	(39%)	1,443	(3%)	14.3
	4	32,271	(39%)	29,977	(36%)	2,294	(3%)	13.1
Inosine	2a)	14,397		7,863		6,534		1.2
(+ Adenosine)	b)	21,891		12,314		9,577		1.3
		18,144	(44%)	10,089	(24%)	8,055	(19%)	1.3
	4[f]	8,881	(25%)	5,565	(16%)	3,316	(9%)	1.7
Inosine	2a)	9,507		9,276		231		40.1
(+ Guanosine)	b)	10,731		10,444		287		36.4
		10,119	(27%)	9,860	(27%)	259	(0.7%)	38.3
	4	61,518	(43%)	47,980	(33%)	13,538	(9%)	3.5

a) 0.5 mM allopurinol added

b) 1 mM aminoimidazolecarboxamide added

c) Percentages of Total Amount Metabolized (from Tables IV-2 and V-3

d) Underlined numbers are the means of two or three preceding samples

e) No measurable radioactivity

f) All larvae in this sample were dead

Table A-5a

Catabolism of Nucleotides to Nucleosides and Bases in Wild-Type Larvae

		Counts per minute/μg DNA					
Precursor	Day and Sample	Total Nucleotides Catabolized	Adenine + Adenosine	Hypoxanthine + Inosine	Guanine + Guanosine	Xanthosine	Succinyl Adenosine
Adenosine	2	2,750^c (1%)^d	-^{f}	-	2,750 (1%)	-	g
	4a)	2,079^c	-	-	2,079	-	g
	b)	937^c	-	-	937	-	g
		1,508^e (1%)			1,508 (1%)		
Hypoxanthine^a	2a)	3,984	1,989	1,139^h	411	72	372
	b)	3,453	867	1,293^h	687	60	547
		3,719 (11%)	1,428 (4%)	1,216^h (4%)	549 (2%)	66 (0.2%)	459 (1%)
	4a)	5,798	1,370	2,135^h	944	400	949
	b)	6,702	4,646	772^h	684	20	580
		6,250 (7%)	3,008 (3%)	1,453^h (2%)	814 (0.9%)	210 (0.2%)	765 (0.9%)
Inosine Exp I	2a)	2,050	944	-	1,055	j	-
	b)	2,239	694	-	1,544	j	-
		2,145 (3%)	819 (1%)		1,299 (2%)		
	4a)	6,999	4,569	-	2,429	j	-
	b)	6,582	4,244	-	2,338	j	-
	c)	8,476	5,469	-	3,007	j	-
		7,352 (4%)	4,761 (3%)		2,591 (1%)		
Inosine Exp II	2	581 (1%)	155 (0.3%)	-	218 (0.4%)	105 (0.2%)	105 (0.1%)
	4	3,069 (2%)	844 (0.6%)	-	1,181 (0.8%)	644 (0.4%)	400 (0.2%)
Guanine	2a)	7,972	517	2,365	4,021^i	841	227
	b)	4,800	347	2,082	1,747^i	323	300
		6,386 (11%)	432 (0.7%)	2,223 (4%)	2,884^i (5%)	582 (1%)	263 (0.4%)
	4	6,186 (18%)	698 (2%)	3,195 (9%)	1,623^i (5%)	318 (0.9%)	352 (1%)
Guanosine	2a)	1,875	496	756	-	306	316
	b)^k	2,905	877	653	-	589	786
	c)	2,785	955	385	-	690	755
		2,522 (4%)	776 (1%)	598 (1%)		528 (0.9%)	619 (1%)
	4	7,769 (10%)	1,200 (2%)	5,169 (7%)	-	1,200 (2%)	200 (0.3%)

Table A-5b

Catabolism of Nucleotides to Nucleosides and Bases in Wild-Type Larvae

Precursor (+ Supplement)	Day and Sample	Total Nucleotides Catabolized	Adenine + Adenosine	Hypoxanthine + Inosine	Xanthine + Xanthosine	Guanine + Guanosine	Uric Acid	Allantoin	Succinyl Adenosine
Formate^b	2a)	13,295	1,870	3,385	85^j	2,230	905	4,820^c	g
Exp I	b)	9,533	967	3,044	155^j	1,244	1,405	2,672^c	g
		11,414 (17%)	1,419 (2%)	3,215 (5%)	120^j (0.2%)	1,737 (3%)	1,177 (2%)	3,746^c (5%)	g
	4	9,807 (9%)	2,000 (2%)	3,505 (3%)	36^j (0.03%)	2,141 (2%)	439 (0.4%)	1,686^c (2%)	g
Formate^b	2a)	5,961	272	333	83	572	833	3,867	g
Exp II	b)	6,085	284	316	42	831	1,021	3,589	g
		6,023 (7%)	278 (0.3%)	325 (0.4%)	63 (0.1%)	701 (0.9%)	927 (1%)	3,728 (5%)	
	4a)	7,295	694	533	205	1,022	428	4,411	g
	b)	6,860	473	653	93	493	227	4,920	g
		7,077 (9%)	583 (0.7%)	593 (0.7%)	149 (0.2%)	757 (0.9%)	327 (0.4%)	4,665 (5%)	
Formate^b	2a)	11,218	300	948	1,948	500	2,835	4,687	g
(+ Guanosine)	b)	11,355	215	350	1,630	360	4,735	4,065	g
		11,287 (24%)	257 (0.5%)	649 (1%)	1,789 (4%)	430 (0.9%)	3,785 (8%)	4,376 (9%)	
	4	18,420 (22%)	485 (0.6%)	4,260 (5%)	4,490 (5%)	370 (0.4%)	2,610 (3%)	6,205 (7%)	g

Inosine	2a)	1,235		430		-[f]	105[l]		595		-	-	105	
(+ Adenosine)	b)	1,752		159		-	345[l]		1,207		-	-	41	
		1,493	(4%)	295	(0.7%)		225[l]	(0.5%)	901	(2%)			73	(0.2%)
	4[k]	3,463	(10%)	627	(2%)	-	378[l]	(1%)	2,107	(6%)	-	-	351	(1%)
Inosine	2a)	725		119		-	125[l]		350		-	-	131	
(+ Guanosine)	b)	578		104		-	104[l]		283		-	-	87	
		652	(2%)	111	(0.3%)		115[l]	(0.3%)	317	(0.9%)			109	(0.3%)
	4	3,583	(2%)	1,583	(1%)	-	483[l]	(0.3%)	1,000	(0.7%)	-	-	517	(0.4%)

a) 0.5 mM allopurinol added

b) 1 mM aminoimidazolecarboxamide added

c) Excluding a small amount in xanthosine

d) Percentages of Total Amount Metabolized (from Tables IV-2 and V-3)

e) Underlined numbers are the means of two or three preceding samples

f) Dashes indicate compounds which are considered direct catabolites of precursor rather than of nucleotides

g) Not measured

h) Inosine only

i) Guanosine only

j) Xanthine only

k) All larvae in this sample were dead

l) Xanthosine only

Table A-6

Distribution of Radioactivity Recovered in *pur1-1* Larvae

Precursor (+ Supplement)	Day and Sample	Counts per minute/ug DNA				
		Total Radioactivity Recovered	Unused Precursor	Total Amount Metabolized	Amount Converted to Nucleotides	Amount of Precursor Catabolized
Glycine	2a)	262,906	158,722	104,184	104,184	e
	b)	246,880	149,429	97,451	97,451	e
		254,893[c]	154,076	100,817 (40%)[d]	100,817	
	4	569,785	357,668	212,117 (37%)	212,117	e
Formate[b]	2a)	149,269	59,582	89,687	89,687	e
	b)	123,049	57,758	65,291	65,291	e
		136,159	58,670	77,489 (57%)	77,489	
	4	114,564	46,996	67,568 (59%)	67,568	e
Hypoxanthine[a]	2	84,263	52,524	31,739 (38%)	30,452	1287
	4a)	127,480	69,347	58,133	56,460	1673
	b)	104,420	60,962	43,458	42,211	1247
		115,950	65,155	50,795 (44%)	49,335	1460
Formate[b]	2	282,292	167,127	115,165 (41%)	115,165	e
(+ Guanosine)	4	262,825	155,498	107,327 (41%)	107,327	e

a) 0.5 mM allopurinol added

b) 1 mM aminoimidazolecarboxamide added

c) Underlined numbers are the means of the two or three preceding samples

d) Percentages of Total Radioactivity Recovered

e) Not measured

Table A-7

Metabolism of Radioactive Nucleotides in *pur1-1* Larvae

Precursor (+ Supplement)	Day and Sample	Counts per minute/µg DNA			
		Amount of Conversion to Nucleotides	Amount in Nucleic Acids	Amount in Acid-Soluble Nucleotides	Amount Catabolized
Glycine	2a)	104,184	57,397	35,906	10,881
	b)	97,451	55,327	29,937	12,187
		100,817[c] (100%)[d]	56,362 (56%)	32,921 (33%)	11,534 (11%)
	4	212,117 (100%)	125,537 (59%)	65,480 (31%)	21,100 (10%)
Formate[b]	2a)	89,688	37,306	34,357	18,025
	b)	65,291	31,548	17,780	15,963
		77,489 (100%)	34,427 (44%)	26,069 (34%)	16,994 (22%)
	4	67,568 (100%)	36,741 (54%)	8,773 (13%)	22,055 (33%)
Hypoxanthine[a]	2	30,452 (96%)	11,665 (37%)	15,200 (48%)	3,587 (11%)
	4a)	56,460	24,720	28,033	3,707
	b)	42,211	21,937	18,907	1,367
		49,335 (97%)	23,328 (46%)	23,470 (46%)	2,537 (5%)
Formate[b]	2	115,165 (100%)	46,934 (41%)	47,892 (41%)	20,338 (18%)
(+ Guanosine)	4	107,327 (100%)	30,677 (29%)	52,350 (49%)	24,300 (23%)

a) 0.5 mM allopurinol added

b) 1 mM aminoimidazolecarboxamide added

c) Underlined numbers are the means of the two or three preceding samples

d) Percentages of Total Amount Metabolized (from Table A-6)

Table A-8

Distribution of Radioactivity in Acid-Soluble Nucleotides in *pur1-1* Larvae

Precursor (+ Supplement)	Day and Sample	Counts per minute/μg DNA						Adenine/ Guanine Ratio
		Remained as Acid-Soluble Nucleotides	Adenine Nucleotides	Guanine Nucleotides	IMP	AMPS	XMP	
Glycine	2a)	35,906	17,656	16,544	1,069	e	637	1.1
	b)	29,937	16,070	12,327	1,033	e	507	1.3
		32,921[c] (33%)	16,863 (17%)[d]	14,435 (14%)	1,051 (1%)		572 (0.6%)	1.2
	4	65,480 (31%)	25,860 (12%)	35,890 (17%)	1,525 (0.7%)	1,595 (0.7%)	610 (0.3%)	0.7
Formate[b]	2a)	34,357	28,450	3,719	1,419	e	769	1.2
	b)	17,781	13,829	2,494	929	e	529	1.3
		26,069 (34%)	21,139 (27%)	3,107 (4%)	1,174 (1%)		649 (0.8%)	1.3
	4	8,773 (13%)	6,382 (9%)	1,182 (2%)	773 (1%)	e	436 (0.6%)	1.4
Hypoxanthine[a]	2	15,200 (48%)	12,944 (41%)	1,494 (5%)	437 (1%)	156 (0.5%)	169 (0.5%)	8.7
	4a)	28,034	23,360	3,267	1,000	e	407	7.1
	b)	18,906	16,207	1,653	613	220 (0.5%)	213	9.8
		23,470 (46%)	19,783 (39%)	2,460 (5%)	807 (1%)		310 (0.6%)	8.5
Formate[b]	2	47,893 (41%)	42,431 (37%)	2,954 (3%)	1,923 (2%)	e	585 (0.5%)	14.4
(+ Guanosine)	4	52,350 (49%)	46,150 (43%)	3,450 (3%)	2,175 (2%)	e	575 (0.5%)	13.4

a) 0.5 mM allopurinol added

b) 1 mM aminoimidazolecarboxamide added

c) Underlined numbers are the means of the two or three preceding samples

d) Percentages of Total Amount Metabolized (from Table A-6)

e) Not measured

Table A-9

Incorporation of Precursor into Nucleic Acid by *pur1-1* Larvae

Precursor (+ Supplement)	Day and Sample	Counts per minute/ug DNA			Adenine/Guanine Ratio
		Total Incorporation into Nucleic Acid	Nucleic Acid Adenine	Nucleic Acid Guanine	
Glycine	2a)	57,397	50,455	6,948	7.3
	b)	55,327	48,320	7,007	6.9
		56,362 (56%)	49,387 (49%)	6,977 (7%)	7.1
	4	125,537 (59%)	112,099 (53%)	13,437 (6%)	8.3
Formate[b]	2a)	37,306	24,287	13,019	1.9
	b)	31,548	19,846	11,701	1.7
		34,427[c] (44%)[d]	22,067 (28%)	12,360 (16%)	1.8
	4	36,741 (54%)	22,525 (33%)	14,216 (21%)	1.6
Hypoxanthine[a]	2	11,665 (37%)	6,120 (19%)	5,545 (17%)	1.1
	4a)	24,720	12,682	12,037	1.1
	b)	21,937	12,209	9,728	1.25
		23,328 (46%)	12,445 (25%)	10,883 (21%)	1.2
Formate[b]	2	46,934 (41%)	46,934 (41%)	[e]	
(+ Guanosine)	4	30,677 (29%)	29,509 (27%)	1,167 (1%)	25.3

a) 0.5 mM allopurinol added

b) 1 mM aminoimidazolecarboxamide added

c) Underlined numbers are the means of the two or three preceding samples

d) Percentages of Total Amount Metabolized (from Table A-6)

e) No measurable radioactivity

Table A-10

Catabolism of Nucleotides to Nucleosides and Bases in *purl-1* Larvae

Precursor (+ Supplement)	Day and Sample	Counts per minute/µg DNA							
		Total Nucleotides Catabolized	Adenine + Adenosine	Hypoxanthine + Inosine	Xanthine + Xanthosine	Guanine + Guanosine	Uric Acid	Allantoin	Succinyl Adenosine
Glycine	2a)	10,881	156	1,419	1,250	856	6,256	944	e
	b)	12,187	127	500	2,307	907	7,333	1,013	e
		11,534[c] (11%)[d]	141 (0.1%)	959 (0.9%)	1,779 (2%)	881 (0.9%)	6,795 (7%)	978 (1%)	e
	4	21,100 (10%)	370 (0.2%)	1,540 (0.7%)	5,955 (3%)	1,195 (0.6%)	7,755 (4%)	4,285 (2%)	e
Formate[b]	2a)	18,025	513	575	2,300	675	9,913	4,050	e
	b)	15,963	217	311	1,697	360	10,763	2,614	e
		16,994 (22%)	365 (0.5%)	443 (0.6%)	1,999 (3%)	517 (0.7%)	10,338 (13%)	3,332 (4%)	e
	4	22,055 (33%)	2,100 (3%)	1,336 (2%)	609 (0.9%)	1,736 (3%)	10,155 (15%)	6,118 (9%)	e
Hypoxanthine[a]	2	3,588 (11%)	2,087 (7%)	463[g] (1%)	119[h] (0.4%)	675 (2%)	-[f]	-	244 (0.8%)
	4a)	3,707	1,987	1,020[g]	33[h]	460	-	-	207
	b)	1,366	320	633[g]	20[h]	260	-	-	133
		2,537 (5%)	1,153 (2%)	827[g] (2%)	27[h] (0.05%)	360 (0.7%)	-	-	170 (0.3%)
Formate[b]	2	20,338 (18%)	646 (0.5%)	969 (0.8%)	1,615 (1%)	985 (0.9%)	10,415 (9%)	5,708 (5%)	e
(+ Guanosine)	4	24,300 (23%)	800 (0.7%)	1,800 (2%)	2,500 (2%)	1,875 (2%)	2,325 (2%)	15,000 (14%)	e

a) 0.5 mM allopurinol added

b) 1 mM aminoimidazolecarboxamide added

c) Underlined numbers are the means of the two or three preceding samples

d) Percentages of Total Amount Metabolized (from Table A-6)

e) Not measured

f) Dashes indicate compounds which are considered catabolites of precursor rather than of nucleotides

g) Inosine only

h) Xanthosine only

Table A-11

Distribution of Radioactivity Recovered in *pur1-2* Larvae

		Counts per minute/μg DNA				
Precursor (+ Supplement)	Day and Sample	Total Radioactivity Recovered	Unused Precursor	Total Amount Metabolized	Amount Converted to Nucleotides	Amount of Precursor Catabolized
Glycine	2	455,217	318,300	136,917 (30%)[c]	136,917	f
	4	635,477	382,715	252,762 (40%)	252,762	f
Formate[b]	2a)	113,144	47,322	65,822	65,822	f
	b)[d]	79,186	28,637	50,549	50,549	f
		96,165[e]	37,979	58,185 (61%)	58,185	
	4	98,950	45,013	53,937 (55%)	53,937	f
Hypoxanthine[a]	2a)	135,706	97,437	38,269	36,716	1,553
	b)	119,886	91,971	27,915	26,939	976
		127,796	94,704	33,092 (26%)	31,827	1,265
	4	295,861	172,091	123,770 (42%)	120,436	3,333
Formate[b]	2a)	212,064	90,835	121,229	121,229	f
(+ Guanosine)	b)	105,547	43,054	62,493	62,493	f
		158,805	66,945	91,861 (58%)	91,861	
	4	341,800	150,297	191,503 (56%)	191,503	f

a) 0.5 mM allopurinol added

b) 1 mM aminoimidazolecarboxamide added

c) Percentages of Total Radioactivity Recovered

d) All larvae in this sample were dead

e) Underlined numbers are the means of two or three preceding samples

f) Not measured

Table A-12

Metabolism of Radioactive Nucleotides in *pur1-2* Larvae

Precursor (+ Supplement)	Day and Sample	Counts per minute/μg DNA			
		Amount of Conversion to Nucleotides	Amount in Nucleic Acids	Amount in Acid-Soluble Nucleotides	Amount Catabolized
Glycine	2	136,917 (100%)[c]	89,508 (65%)	30,158 (22%)	17,251 (13%)
	4	252,762 (100%)	140,500 (55%)	70,693 (28%)	41,569 (16%)
Formate[b]	2a)	65,822	36,497	15,519	13,806
	b)[d]	50,549	26,532	12,741	11,276
		58,185[e] (100%)	31,515 (54%)	14,130 (24%)	12,541 (22%)
	4	53,937 (100%)	27,625 (51%)	12,125 (23%)	14,188 (26%)
Hypoxanthine[a]	2a)	36,716	15,669	19,294	1,753
	b)	26,939	10,782	13,828	2,329
		31,827 (96%)	13,225 (40%)	16,561 (50%)	2,041 (6%)
	4	120,436 (97%)	50,653 (41%)	58,050 (47%)	11,733 (9%)
Formate[b] (+ Guanosine)	2a)	121,229	43,800	48,143	29,286
	b)	62,493	21,497	25,020	15,976
		91,861 (100%)	32,649 (36%)	36,581 (40%)	22,631 (24%)
	4	191,503 (100%)	47,503 (25%)	90,633 (47%)	53,367 (28%)

a) 0.5 mM allopurinol added

b) 1 mM aminoimidazolecarbozamide

c) Percentages of Total Amount Metabolized (from Table A-11)

d) All larvae in this sample were dead

e) Underlined numbers are the means of two or three preceding samples

Table A-13

Distribution of Radioactivity in Acid-Soluble Nucleotides in purl-2 Larvae

		Counts per minute/ug DNA						
Precursor (+ Supplement)	Day and Sample	Remained as Acid-Soluble Nucleotides	Adenine Nucleotides	Guanine Nucleotides	IMP	AMPS	XMP	Adenine/Guanine Ratio
Glycine	2	30,159 (22%)[c]	18,058 (13%)	10,292 (7%)	1,167 (0.9%)	200 (0.1%)	442 (0.3%)	1.8
	4	70,693 (28%)	35,231 (14%)	30,323 (12%)	2,631 (1%)	1,185 (0.5%)	1,323 (0.5%)	1.2
Formate[b]	2a)	15,519	11,350	2,619	1,087	f	463	4.3
	b)[d]	12,741	9,031	2,048	1,276	f	386	4.4
		14,130[e] (24%)	10,191 (17%)	2,333 (4%)	1,181 (2%)		425 (0.7%)	4.3
	4	12,125 (23%)	10,375 (19%)	850 (1%)	425 (0.8%)	f	475 (0.9%)	12.2
Hypoxanthine[a]	2a)	19,294	15,794	2,735	665	f	100	5.8
	b)	13,829	11,490	1,867	405	f	67	6.1
		16,561 (50%)	13,642 (41%)	2,301 (7%)	535 (2%)		83 (0.3%)	5.9
	4	58,049 (47%)	44,100 (36%)	11,927 (10%)	1,828 (1%)	f	194 (0.1%)	3.7
Formate[b]	2a)	48,143	35,136	4,150	7,293	f	1,564	8.5
(+ Guanosine)	b)	25,019	16,693	2,853	4,643	f	830	5.9
		36,581 (40%)	25,915 (28%)	3,501 (4%)	5,968 (6%)		1,197 (1%)	7.2
	4	90,634 (47%)	66,667 (35%)	11,700 (6%)	9,100 (5%)	f	3,167 (2%)	5.7

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a) 0.5 mM allopurinol added

b) 1 mM aminoimidazolecarboxamide added

c) Percentages of Total Amount Metabolized (from Table A-11)

d) All larvae in this sample were dead

e) Underlined numbers are the means of two or three preceding samples

f) Not measured

Table A-14

Incorporation of Precursor into Nucleic Acid by *pur1-2* Larvae

Precursor (+ Supplement)	Day and Sample	Counts per minute/µg DNA Total Incorporation into Nucleic Acid	Nucleic Acid Adenine	Nucleic Acid Guanine	Adenine/Guanine Ratio
Glycine	2	89,508 (65%)[c]	77,764 (57%)	11,744 (9%)	6.6
	4	140,500 (55%)	122,174 (48%)	18,327 (7%)	6.7
Formate[b]	2a)	36,497	23,672	12,825	1.8
	b)[d]	26,532	17,551	8,981	1.9
		31,515[e] (54%)	20,611 (35%)	10,903 (19%)	1.9
	4	27,625 (51%)	16,605 (31%)	11,020 (20%)	1.5
Hypoxanthine[a]	2a)	15,669	9,748	5,921	1.7
	b)	10,782	6,267	4,515	1.4
		13,225 (40%)	8,007 (24%)	5,218 (16%)	1.5
	4	50,653 (41%)	31,071 (25%)	19,582 (16%)	1.6
Formate[b]	2a)	43,800	41,838	1,962	21.3
(+ Guanosine)	b)	21,497	20,021	1,475	13.6
		32,649 (36%)	30,929 (34%)	1,719 (2%)	17.5
	4	47,503 (25%)	42,443 (22%)	5,059 (3%)	8.4

a) 0.5 mM allopurinol added

b) 1 mM aminoimidazolecarboxamide added

c) Percentages of Total Amount Metabolized (from Table A-11)

d) All larvae in this sample were dead

e) Underlined numbers are the means of two or three preceding samples

Table A-15

Catabolism of Nucleotides to Nucleosides and Bases in *purl-2* Larvae

Precursor (+ Supplement)	Day and Sample	Total Nucleotides Catabolized	Adenine + Adenosine	Hypoxanthine + Inosine	Xanthine + Xanthosine	Guanine + Guanosine	Uric Acid	Allantoin	Succinyl Adenosine
		Counts per minute/ug DNA							
Glycine	2	17,251 (13%)^{c}	300 (0.2%)	1,092 (0.8%)	4,092 (3%)	7,509 (5%)	2,275 (2%)	1,983 (1%)	h
	4	41,569 (16%)	754 (0.3%)	4,092 (2%)	8,800 (3%)	5,508 (2%)	4,092 (2%)	18,323 (7%)	h
Formate^{b}	2a)	13,806	181	469	1,656	631	3,306	7,563	h
	b)^{d}	11,276	338	524	1,138	1,076	1,752	6,448	h
		12,541^{e} (22%)	259 (0.4%)	497 (0.8%)	1,397 (2%)	853 (1%)	2,529 (4%)	7,005 (12%)	h
	4	14,188 (26%)	4,287 (8%)	1,300 (2%)	255 (0.4%)	3,300 (6%)	1,363 (3%)	3,713 (7%)	h
Hypoxanthine^{a}	2a)	1,753	294	571^{f}	100^{g}	235	-^{i}	-	553
	b)	2,328	1,343	419^{f}	76^{g}	238	-	-	252
		2,041 (6%)	819 (3%)	495^{f} (1%)	88^{g}(0.3%)	237 (0.7%)	-	-	403 (1%)
	4	11,732 (9%)	8,555 (7%)	1,511^{f} (1%)	333^{g}(0.3%)	455 (0.4%)	-	-	878 (0.7%)
Formate^{b}	2a)	29,286	986	750	3,664	700	6,607	16,579	h
(+ Guanosine)	b)	15,976	357	310	2,483	203	3,520	9,103	h
		22,631 (24%)	671 (0.7%)	530 (0.6%)	3,073 (3%)	451 (0.5%)	5,063 (5%)	12,841 (14%)	h
	4	53,367 (28%)	667 (0.3%)	1,633 (0.8%)	15,667 (8%)	3,900 (2%)	15,867 (8%)	15,633 (8%)	h

a) 0.5 mM allopurinol added

b) 1 mM aminoimidazolacarboxamide added

c) Percentages of Total Amount Metabolized (from Table A-11)

d) All larvae in this sample were dead

e) Underlined numbers are the means of two or three preceding samples

f) Inosine only

g) Xanthosine only

h) Not measured

i) Dashes indicate compounds which are considered direct catabolites of precursor rather than of nucleotides

Table A-16

Distribution of Radioactivity Recovered in *ade2-1* Larvae

Precursor (+ Supplement)	Day and Sample	Counts per minute/μg DNA				
		Total Radioactivity Recovered	Unused Precursor	Total Amount Metabolized	Amount Converted to Nucleotides	Amount of Precursor Catabolized
Glycine	2	238,540	163,096	75,444 (32%)[c]	75,444	e
	4a)	668,487	527,972	140,515	140,515	e
	b)	539,842	416,682	123,160	123,160	e
		604,165[d]	472,327	131,838 (22%)	131,838	
Formate[b]	2a)	76,384	34,067	42,317	42,317	e
	b)	103,108	47,137	55,971	59,971	e
		89,746	40,602	49,144 (55%)	49,144	
	4	113,555	46,448	67,107 (59%)	67,107	e
Hypoxanthine[a]	2a)	274,560	207,247	67,313	62,113	5,200
	b)	264,169	218,919	45,250	40,573	4,677
		269,365	213,083	56,282 (21%)	51,343	4,939
	4a)	108,110	70,377	37,733	35,883	1,850
	b)	139,413	93,367	46,046	42,971	3,075
		123,761	81,872	41,889 (34%)	39,427	2,463

Guanine	2a)	124,553	87,299	37,254		14,938	24,820
	b)	163,669	134,561	29,108		11,864	17,244
		144,111	110,930	33,181	(23%)	13,401	21,032
	4	64,305	33,338	30,967	(48%)	15,624	15,343
Guanosine	2a)	208,775	59,904	148,871		97,971	50,900
	b)	125,440	13,789	111,651		83,000	28,651
		167,107	36,847	130,261	(78%)	90,485	39,775
	4a)	165,989	23,671	142,318		89,012	53,305
	b)	193,886	47,549	146,337		76,609	69,729
		179,937	35,610	144,327	(80%)	82,811	61,517
Formate[b]	2a)	100,036	55,595	44,441		44,441	e
(+ Adenosine)	b)[f]	95,025	45,222	49,803		49,803	e
		97,530	50,409	47,122	(48%)	47,122	
	4	386,567	170,245	216,322	(56%)	216,322	e

a) 0.5 mM allpurinol added

b) 1 mM aminoimidazolecarboxamide added

c) Percentages of Total Radioactivity Recovered

d) Underlined numbers are the means of the two or three preceding samples

e) Not measured

f) All larvae in this sample were dead

Table A-17

Metabolism of Radioactive Nucleotides in *ade2-1* Larvae

Precursor (+ Supplement)	Day and Sample	Counts per minute/μg DNA			
		Total Conversion to Nucleotides	Amount in Nucleic Acids	Amount in Acid-Soluble Nucleotides	Amount Catabolized
Glycine	2	75,444 (100%)[c]	31,425 (42%)	32,366 (43%)	11,653 (15%)
	4a)	140,515	85,239	45,590	9,686
	b)	123,160	74,561	42,299	6,300
		131,838[d] (100%)	79,900 (60%)	43,945 (33%)	7,993 (6%)
Formate[b]	2a)	42,317	20,822	14,605	6,889
	b)	55,971	17,848	26,769	11,354
		49,143 (100%)	19,335 (39%)	20,687 (42%)	9,121 (19%)
	4	67,107 (100%)	33,175 (49%)	22,800 (34%)	11,132 (17%)
Hypoxanthine[a]	2a)	62,113	21,669	33,440	7,004
	b)	40,573	7,989	26,861	5,723
		51,343 (91%)	14,829 (26%)	30,151 (54%)	6,364 (11%)
	4a)	35,883	18,303	15,920	1,760
	b)	42,971	20,909	18,975	3,089
		39,427 (94%)	19,606 (47%)	17,447 (42%)	2,424 (6%)

Guanine	2a)	14,938		1,824		2,127		10,987	
	b)	11,864		339		1,425		10,100	
		13,401	(40%)	1,081	(3%)	1,776	(5%)	10,543	(32%)
	4	15,624	(50%)	382	(1%)	1,719	(5%)	13,524	(44%)
Guanosine	2a)	97,971		74,596		18,117		5,258	
	b)	83,000		62,714		17,857		2,429	
		90,535	(69%)	68,655	(53%)	18,037	(13%)	3,843	(3%)
	4a)	89,012		70,644		10,326		8,042	
	b)	76,609		60,066		7,257		9,286	
		82,811	(57%)	65,355	(45%)	8,791	(6%)	8,664	(6%)
Formate[b]	2a)	44,441		11,384		14,271		18,786	
(+ Adenosine)	b)[e]	49,803		9,627		13,058		27,117	
		47,122	(100%)	10,505	(22%)	13,665	(29%)	22,951	(49%)
	4	216,322	(100%)	74,438	(34%)	55,050	(25%)	86,833	(40%)

a) 0.5 mM allopurinol added

b) 1 mM aminoimidazolecarboxamide added

c) Percentages of Total Amount Metabolized (from Table A-16)

d) Underlined numbers are the means of the two or three preceding samples

e) All larvae in this sample were dead

Table A-18

Distribution of Radioactivity in Acid-Soluble Nucleotides in *ade2-1* Larvae

		Counts per minute/µg DNA						
Precursor (+ Supplement)	Day and Sample	Remained as Acid-Soluble Nucleotides	Adenine Nucleotides	Guanine Nucleotides	IMP	AMPS	XMP	Adenine/Guanine Ratio
Glycine	2	32,366 (43%)[c]	23,913 (32%)	5,153 (7%)	1,687 (2%)	700 (0.9%)	913 (1%)	4.6
	4a)	45,590	33,887	7,100	1,925	1,787	887	4.8
	b)	42,299	29,550	8,358	2,483	958	950	3.5
		43,945[d] (33%)	31,719 (24%)	7,729 (6%)	2,054 (1%)	1,373 (1%)	919 (0.7%)	4.1
Formate[b]	2a)	14,604	11,068	2,700	421	268	147	4.1
	b)	26,769	19,708	5,477	738	415	431	3.6
		20,687 (42%)	15,388 (31%)	4,089 (8%)	579 (1%)	341 (0.7%)	289 (0.6%)	3.8
	4	22,800 (34%)	14,623 (22%)	5,627 (8%)	1,091 (2%)	914 (1%)	545 (0.8%)	2.6
Hypoxanthine[a]	2a)	33,440	27,940	3,620	1,220	400	260	7.7
	b)	26,861	22,031	3,215	1,185	292	138	6.9
		30,151 (54%)	24,985 (44%)	3,417 (6%)	1,203 (2%)	346 (0.6%)	199 (0.3%)	7.3
	4a)	15,920	12,940	1,740	850	290	100	7.4
	b)	18,975	14,787	2,637	1,175	263	113	5.6
		17,447 (42%)	13,863 (33%)	2,189 (5%)	1,013 (2%)	277 (0.7%)	107 (0.3%)	6.5

Guanine	2a)	2,127	373	1,027	320	60	347	0.36
	b)	1,425	350	144	719	69	144	2.4
		1,776 (5%)	361 (1%)	585 (2%)	519 (1%)	65 (0.2%)	245 (0.7%)	1.4
	4	1,719 (5%)	424 (1%)	495 (2%)	252 (0.8%)	100 (0.3%)	448 (1%)	0.86
Guanosine	2a)	18,116	1,342	14,758	1,108	183	725	0.09
	b)	17,857	491	16,891	211	114	149	0.03
		18,037 (13%)	917 (0.7%)	15,825 (12%)	659 (0.5%)	149 (0.1%)	437 (0.3%)	0.06
	4a)	10,327	895	7,400	516	421	1,095	0.12
	b)	7,257	586	4,214	971	129	1,357	0.14
		8,791 (6%)	741 (0.5%)	5,807 (4%)	743 (0.5%)	275 (0.2%)	1,226 (0.8%)	0.13
Formate^b	2a)	14,272	10,925	2,429	446	261	211	4.5
(+ Adenosine)	b)^e	13,058	9,316	2,667	600	192	283	3.5
		13,665 (29%)	10,121 (21%)	2,548 (5%)	523 (1%)	227 (0.5%)	247 (0.5%)	4.0
	4	55,050 (25%)	38,600 (18%)	10,567 (5%)	3,950 (2%)	683 (0.3%)	1,250 (0.6%)	3.7

a) 0.5 mM allopurinol added

b) 1 mM aminoimidazolecarboxamide added

c) Percentages of Total Amount Metabolized (from Table A-16)

d) Underlined numbers are the means of the two or three preceding samples

e) All larvae in this sample were dead

Table A-19

Incorporation of Precursor into Nucleic Acid by *ade2-1* Larvae

Precursor (+ Supplement)	Day and Sample	Counts per minute/μg DNA			Adenine/Guanine Ratio
		Total Incorporation into Nucleic Acid	Nucleic Acid Adenine	Nucleic Acid Guanine	
Glycine	2	31,425 (42%)[c]	30,305 (40%)	1,120 (1%)	27.1
	4a)	85,239	80,094	5,146	15.6
	b)	74,561	70,436	4,125	17.1
		79,900[d] (37%)	75,265 (34%)	4,635 (2%)	16.3
Formate[b]	2a)	20,822	12,839	7,983	1.61
	b)	17,848	11,005	6,843	1.61
		19,335 (39%)	11,922 (24%)	7,413 (15%)	1.61
	4	33,175 (49%)	21,877 (33%)	11,299 (17%)	1.94
Hypoxanthine[a]	2a)	21,669	15,196	6,473	2.35
	b)	7,989	4,728	3,261	1.45
		14,829 (26%)	9,962 (18%)	3,303 (8%)	1.9
	4a)	18,303	11,770	6,533	1.80
	b)	20,909	13,440	7,469	1.79
		19,606 (47%)	12,605 (30%)	7,001 (17%)	1.8

Guanine	2a)	1,824		105		1,719		0.06
	b)	339		56		283		0.20
		1,081	(3%)	81	(0.2%)	1,001	(3%)	0.13
	4	382	(1%)	71	(0.2%)	312	(1%)	0.23
Guanosine	2a)	74,596		e		74,596		
	b)	62,714		e		62,714		
		68,655	(53%)			68,655	(53%)	
	4a)	70,644		e		70,644		
	b)	60,066		e		60,065		
		65,355	(45%)			65,355	(45%)	
Formate[b]	2a)	11,384		8,048		3,337		2.41
(+ Adenosine)	b)[f]	9,627		7,511		2,117		3.55
		10,505	(22%)	7,779	(17%)	2,727	(6%)	2.98
	4	74,438	(34%)	54,470	(25%)	19,968	(9%)	2.73

a) 0.5 mM allopurinol added

b) 1 mM aminoimidazolecarboxamide added

c) Percentages of Total Amount Metabolized (from Table A-16)

d) Underlined numbers are the means of the two or three preceding samples

e) No measurable radioactivity

f) All larvae in this sample were dead

Table A-20

Catabolism of Nucleotides to Nucleosides and Bases in *ade2-1* Larvae

Precursor (+ Supplement)	Day and Sample	Counts per minute/µg DNA							
		Total Nucleotides Catabolized	Adenine + Adenosine	Hypoxanthine + Inosine	Xanthine + Xanthosine	Guanine + Guanosine	Uric Acid	Allantoin	Succinyl Adenosine
Glycine	2	11,653 (15%)[c]	267 (0.3%)	1,073 (1%)	333 (0.4%)	1,693 (2%)	3,947 (5%)	4,340 (6%)	•
	4a)	9,686	1,937	5,537	263	•	462	1,487	•
	b)	6,300	767	1,575	217	•	258	3,483	•
		7,993[d] (6%)	1,352 (1%)	3,556 (3%)	240 (0.2%)		360 (0.3%)	2,485 (2%)	
Formate[b]	2a)	6,889	131	531	811	2,289	442	2,684	•
	b)	11,354	308	615	1,061	7,585	585	1,200	•
		9,121 (19%)	219 (0.4%)	573 (1%)	936 (2%)	4,937 (10%)	513 (1%)	1,942 (4%)	
	4	11,132 (17%)	668 (1%)	413 (0.6%)	2,231 (3%)	6,664 (10%)	318 (0.5%)	836 (1%)	•
Hypoxanthine[a]	2a)	7,004	2,804	1,760[f]	120[g]	1,280	-[j]	-	1,040
	b)	5,723	2,708	954[f]	154[g]	1,046	-	-	861
		6,364 (11%)	2,756 (5%)	1,357[f] (2%)	137[g] (0.2%)	1,163 (2%)			951 (2%)
	4a)	1,760	540	420[f]	120[g]	350	-	-	330
	b)	3,089	913	713[f]	487[g]	513	-	-	463
		2,424 (6%)	727 (2%)	567[f] (1%)	303[g] (0.7%)	431 (1%)			396 (0.9%)

Guanine	2a)	10,987	387	5,220	567^g	4,393^h	-	-	420
	b)	10,101	463	4,175	200^g	4,550^h	-	-	713
		10,544 (32%)	425 (1%)	4,697 (14%)	383^g (1%)	4,471^h (13%)			561 (2%)
	4	13.524 (44%)	495 (1%)	9,533 (31%)	1,043^g (3%)	2,333^h (7%)	-	-	119 (0.4%)
Guanosine	2a)	5,258	1,358	3,250	317^g	-	-	-	333
	b)	2,428	1,491	354	354^g	-	-	-	229
		3,843 (3%)	1,424 (1%)	1,802 (1%)	335^g(0.3%)				281 (0.2%)
	4a)	8,042	3,074	4,295	347^g	-	-	-	326
	b)	9,285	3,171	5,429	214^g	-	-	-	471
		8,663 (6%)	3,123 (2%)	4,862 (3%)	281^g(0.2%)				399 (0.3%)
Formate^b	2a)	18,786	214	3,464	246	9,821	3,136	1,903	e
(+ Adenosine)	b)^i	27,117	308	1,975	467	12,050	5,108	7,208	e
		22,951 (49%)	261 (0.5%)	2,719 (6%)	357 (0.7%)	10,935 (23%)	4,122 (9%)	4,555 (10%)	e
	4	86,833 (40%)	1,650 (0.8%)	4,250 (2%)	967 (0.4%)	47,067 (22%)	11,200 (5%)	21,700 (10%)	e

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a) 0.5 mM allopurinol added

b) 1 mM aminoimidazolecarboxamide added

c) Percentages of Total Amount Metabolized (from Table A-16)

d) Underlined numbers are the means of the two or three preceding samples

e) Not measured

f) Inosine only

g) Xanthosine only

h) Guanosine only

i) All larvae in this sample were dead

j) Dashes indicate compounds which are catabolites of precursor rather than of nucleotides

Table A-21

Precursor Catabolism in *pur1-1*, *pur1-2*, and *ade2-1* Larvae

Mutant	Precursor	Day and Sample	Total Amount Catabolized	Guanine	Xanthine	Uric Acid	Allantoin
			Counts per minute/μg DNA				
pur1-1	Hypoxanthine[a]	2	1,287 (4%)[b]	-[d]	806 (3%)	119 (0.4%)	363 (1%)
		4a)	1,673	-	1,013	53	607
		b)	1,247	-	973	0	273
			1,460[c] (3%)	-	993 (2%)	27 (0.05%)	440 (0.9%)
pur1-2	Hypoxanthine[a]	2a)	1,553	-	1,006	518	29
		b)	976	-	738	229	10
			1,265 (4%)	-	872 (3%)	373 (1%)	19 (0.1%)
		4	3,333 (3%)	-	1,583 (1%)	1,588 (1%)	161 (0.1%)
ade2-1	Hypoxanthine[a]	2a)	5,200	-	4,560	240	400
		b)	4,677	-	3,477	646	554
			4,939 (9%)	-	4,019 (7%)	443 (0.8%)	477 (0.9%)
		4a)	1,850	-	1,660	120	70
		b)	3,075	-	1,900	1,075	100
			2,463 (6%)	-	1,825 (4%)	597 (1%)	85 (0.2%)
	Guanine	2a)	24,820	-	5,207	5,887	13,727
		b)	17,244	-	3,200	3,325	10,719
			21,032 (63%)	-	4,203 (13%)	4,606 (14%)	12,223 (37%)
		4	25,343 (54%)	-	6,124 (21%)	2,190 (8%)	7,029 (25%)
	Guanosine	2a)	30,900	27,642	9,683	5,975	7,600
		b)	28,651	22,520	743	1,189	4,200
			39,775 (31%)	25,081 (19%)	5,213 (4%)	3,582 (3%)	5,900 (5%)
		4a)	53,305	36,463	2,653	2,747	11,442
		b)	69,729	54,857	2,329	3,014	9,529
			61,517 (43%)	45,660 (32%)	2,491 (2%)	2,880 (2%)	10,485 (7%)

a) 0.5 mM allopurinol added

b) Percentages of Total Amount Metabolized (from Tables A-6, A-11, and A-16, for the three mutants)

c) Underlined numbers are the means of the two or three preceding samples

d) Dashes indicate compounds which are not considered precursor catabolites of a particular precursor

Table A-22

Distribution of Radioactivity Recovered in *gua2-1* Larvae

Precursor (+ Supplement)	Day and Sample	Counts per minute/µg DNA Total Radioactivity Recovered	Unused Precursor	Total Amount Metabolized	Amount Converted to Nucleotides	Amount of Precursor Catabolized
Hypoxanthine[a]	2	96,178	64,756	31,422 (33%)[c]	27,489	3,933
	4	168,133	83,714	84,419 (50%)	64,360	20,059
Inosine	2a)	77,600	29,033	48,567	32,992	15,575
	b)	71,255	32,447	38,808	25,225	13,583
		74,427[b]	30,740	43,687 (59%)	29,109	14,579
	4	118,489	54,984	63,505 (53%)	41,517	21,989
Guanosine	2a)	600,017	361,196	238,821	147,612	91,208
	b)[d]	458,485	197,911	260,574	129,733	130,841
		529,251	279,553	249,697 (47%)	138,673	111,025
	4	475,625	194,688	280,937 (59%)	210,854	70,083
Inosine	2	89,806	39,652	50,154 (56%)	31,342	18,812
(+ Guanosine)	4	146,650	52,475	94,175 (64%)	68,025	26,150
Guanine	2	92,864	28,799	64,065 (69%)	16,461	47,604
(+ Guanosine)	4	179,231	108,044	71,187 (40%)	19,781	51,406

a) 0.5 mM allopurinol added

b) Underlined numbers are the means of the two or three preceding samples

c) Percentages of Total Radioactivity Recovered

d) All larvae in this sample were dead

Table A-23

Catabolism of Precursor in *gua2-1* Larvae

Precursor (+ Supplement)	Day and Sample	Counts per minute/µg DNA Total Amount Catabolized	Hypoxanthine	Guanine	Xanthine	Uric Acid	Allantoin
Hypoxanthine[a]	2	3,933 (13%)[b]	-[e]	-	3,859 (12%)	30 (0.09%)	44 (0.1%)
	4	20,059 (24%)	-	-	18,600 (22%)	867 (1%)	592 (0.7%)
Inosine	2a)	15,576	494	-	119	14,469	494
	b)	13,583	422	-	389	11,855	917
		14,579 (33%)	458 (1%)	-	254 (0.6%)	13,162 (30%)	705 (2%)
	4	21,989 (35%)	689 (1%)	-	278 (0.4%)	18,833 (30%)	2,189 (3%)
Guanosine	2a)	91,209	-	1,592	5,942	20,500	63,175
	b)	130,840	-	1,555	8,363	27,855	93,067
		111,025 (44%)	-	1,573 (0.6%)	7,153 (3%)	24,177 (10%)	78,121 (31%)
	4	70,084 (25%)	-	14,542 (5%)	9,646 (3%)	15,792 (6%)	30,104 (11%)
Inosine	2	18,812 (28%)	424 (0.6%)	-	1,303 (2%)	15,267 (22%)	1,818 (3%)
(+ Guanosine)	4	26,150 (17%)	1,600 (1%)	-	750 (0.5%)	19,175 (13%)	4,625 (3%)
Guanine	2	47,604 (88%)	-	-	4,020 (7%)	31,584 (58%)	12,000 (22%)
(+ Guanosine)	4	51,407 (71%)	-	-	21,019 (29%)	23,069 (32%)	7,319 (10%)

a) 0.5 mM allopurinol added

b) Percentages of Total Amount Metabolized (from Table A-22)

c) Underlined numbers are the means of the two or three preceding samples

d) All larvae in this sample were dead

e) Dashes indicate compounds which are not considered precursor catabolites of a particular precursor

Table A-24

Metabolism of Radioactive Nucleotides in *gua2-1* Larvae

Precursor (+ Supplement)	Day and Sample	Counts per minute/µg DNA			
		Amount of Conversion to Nucleotides	Amount in Nucleic Acids	Amount in Acid-Soluble Nucleotides	Amount Catabolized
Hypoxanthine[a]	2	27,489 (87%)[b]	10,637 (34%)	10,837 (34%)	6,015 (19%)
	4	64,360 (76%)	25,125 (30%)	35,592 (42%)	3,643 (4%)
Inosine	2a)	32,992	16,617	15,781	594
	b)	25,225	15,958	8,550	717
		29,109[c] (67%)	16,287 (37%)	12,165 (28%)	655 (1%)
	4	41,517 (65%)	16,417 (26%)	23,878 (38%)	1,222 (2%)
Guanosine	2a)	147,612	86,754	51,283	9,575
	b)[d]	129,733	76,385	41,663	11,685
		138,673 (55%)	81,569 (33%)	46,473 (19%)	10,630 (4%)
	4	210,854 (75%)	153,979 (55%)	26,229 (9%)	30,646 (11%)
Inosine	2	31,342 (62%)	13,337 (27%)	15,721 (31%)	2,284 (5%)
(+ Guanosine)	4	68,025 (72%)	24,825 (26%)	16,475 (17%)	26,725 (28%)
Guanine	2	16,461 (26%)	261 (0.4%)	2,004 (3%)	14,196 (22%)
(+ Guanosine)	4	19,781 (28%)	307 (0.4%)	2,969 (4%)	16,505 (23%)

a) 0.5 mM allopurinol added

b) Percentages of Total Amount Metabolized (from Table A-22)

c) Underlined numbers are the means of the two or three preceding samples

d) All larvae in this sample were dead

Table A-25

Distribution of Radioactivity in Acid-Soluble Nucleotides in *gua2-1* Larvae

Precursor (+ Supplement)	Day and Sample	Counts per minute/µg DNA					Adenine/ Guanine Ratio
		Remained as Acid-Soluble Nucleotides	Adenine Nucleotides	Guanine Nucleotides	IMP	XMP	
Hypoxanthine[a]	2	10,838 (34%)	9,474 (30%)	830 (3%)	393 (1%)	141 (0.4%)	11.4
	4	35,592 (42%)	30,658 (36%)	3,492 (4%)	1,050 (1%)	392 (0.5%)	8.8
Inosine	2a)	15,781	13,506	1,037	969	269	13.0
	b)	8,550	6,933	828	600	189	8.4
		12,165[c] (28%)	10,219 (23%)	933 (2%)	785 (2%)	229 (0.5%)	10.9
	4	23,877 (38%)	19,544 (31%)	2,611 (4%)	1,200 (2%)	522 (0.8%)	7.5
Guanosine	2a)	51,283	7,900	38,233	1,975	3,175	0.21
	b)[d]	41,663	8,085	28,363	2,426	2,789	0.29
		46,473 (19%)	7,993 (3%)	33,298 (13%)	2,201 (0.9%)	2,982 (1%)	0.24
	4	26,230 (9%)	4,375 (1%)	15,521 (5%)	3,521 (1%)	2,813 (1%)	0.28
Inosine	2	15,721 (31%)	14,097 (28%)	739 (1%)	612 (1%)	273 (0.5%)	19.1
(+ Guanosine)	4	16,474 (17%)	14,563 (15%)	1,137 (1%)	337 (0.3%)	437 (0.5%)	12.8
Guanine	2	2,004 (3%)	1,080 (2%)	260 (0.4%)	500 (0.8%)	164 (0.3%)	4.1
(+ Guanosine)	4	2,968 (4%)	1,237 (2%)	856 (1%)	569 (0.8%)	306 (0.4%)	1.4

a) 0.5 mM allopurinol added

b) Percentages of Total Amount Metabolized (from Table A-22)

c) Underlined numbers are the means of the two or three preceding samples

d) All larvae in this sample were dead

Table A-26

Incorporation of Precursor into Nucleic Acid by *gua3-1* Larvae

Precursor (+ Supplement)	Day and Sample	Counts per minute/ug DNA: Total Incorporation into Nucleic Acid	Nucleic Acid Adenine	Nucleic Acid Guanine	Adenine/Guanine Ratio
Hypoxanthine[a]	2	10,637 (34%)[b]	6,463 (21%)	4,174 (13%)	1.5
	4	25,125 (30%)	15,808 (19%)	9,485 (11%)	1.7
Inosine	2a)	16,617	10,974	5,644	1.9
	b)	15,958	10,315	5,643	1.8
		16,287[c] (37%)	10,645 (24%)	5,643 (13%)	1.85
	4	16,417 (26%)	10,363 (16%)	6,053 (10%)	1.7
Guanosine	2a)	86,754	e	86,754	
	b)[d]	76,385	e	76,385	
		81,569 (33%)		81,569 (33%)	
	4	153,979 (55%)	e	153,980 (55%)	
Inosine	2	13,337 (27%)	13,337 (27%)	e	
(+ Guanosine)	4	24,825 (26%)	24,487 (26%)	337 (0.3%)	72.7
Guanine	2	261 (0.4%)	133 (0.2%)	128 (0.2%)	1.0
(+ Guanosine)	4	307 (0.4%)	119 (0.1%)	189 (0.3%)	0.6

a) 0.5 mM allopurinol added

b) Percentages of Total Amount Metabolized (from Table A-22)

c) Underlined numbers are the means of the two or three preceding samples

d) All larvae in this sample were dead

e) No measurable radioactivity

Table A-27

Catabolism of Nucleotides to Nucleosides and Bases in *gua2-1* Larvae

Precursor (+ Supplement)	Day and Sample	Counts per minute/μg DNA					
		Total Nucleotides Catabolized	Adenine + Adenosine	Hypoxanthine + Inosine	Guanine + Guanosine	Xanthosine	Succinyl Adenosine
Hypoxanthine[a]	2	6,015 (19%)[b]	4,570 (15%)	467[c] (1%)	652 (2%)	126 (0.4%)	200 (0.6%)
	4	3,643 (4%)	1,367 (2%)	983[c] (1%)	268 (0.3%)	333 (0.4%)	692 (0.8%)
Inosine	2a)	593	187	e	187	219	h
	b)	717	67	e	489	161	h
		655[d] (1%)	127 (0.3%)		338 (0.8%)	190 (0.4%)	
	4	1,222 (2%)	500 (0.8%)	e	422 (0.7%)	300 (0.5%)	h
Guanosine	2a)	9,575	1,875	2,683	e	1,958	3,058
	b)[g]	11,685	4,678	2,933	e	2,441	1,633
		10,630 (4%)	3,277 (1%)	2,808 (1%)		2,199 (0.9%)	2,345 (0.9%)
	4	30,646 (11%)	4,437 (2%)	21,083 (7%)	e	4,063 (1%)	1,063 (0.4%)
Inosine	2	2,284 (5%)	800 (2%)	e	824 (2%)	242 (0.5%)	418 (0.8%)
(+ Guanosine)	4	26,725 (28%)	25,675 (27%)	e	200 (0.2%)	425 (0.5%)	425 (0.5%)
Guanine	2	14,196 (22%)	552 (0.9%)	2,592 (4%)	2,784[f] (4%)	4,636 (7%)	3,632 (6%)
(+ Guanosine)	4	16,505 (23%)	1,231 (2%)	10,787 (15%)	2,781[f] (4%)	537 (0.7%)	1,169 (2%)

a) 0.5 mM allopurinol added

b) Percentages of Total Amount Metabolized (from Table A-22)

c) Inosine only

d) Underlined numbers are the means of the two or three preceding samples

e) Predominantly precursor

f) Guanosine only

g) All larvae in this sample were dead

h) Not measured